THE BRITISH VETERINARY JOURNAL

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Vol. 113 **April, 1957** **No. 4**

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EDITORIAL

CHANGES IN CIRCUMSTANCES CALL FOR NEW METHODS

THE way of the world today is entirely different from that which was pursued in 1900. If a man who had lived his life in the 19th century could visit again his native place in this year of grace he would be amazed and confounded by the changes that have occurred. Many things that he would see and hear would be far beyond his comprehension, and it would not be strange if he were to think that some Geni with a magic lamp had been at work. He would find extraordinary change in every facet of life, and if he had been a veterinary surgeon he would note that none of the changes which had occurred would be greater than those associated with his own profession.

In times gone by most veterinary surgeons were occupied mainly in the treatment of established disease in individual animals. The theme of their endeavour was to cure. In modern times the emphasis is not on the cure of the individual but on the protection of the many in the stud, herd or flock. It may be necessary to sacrifice the diseased individual so that the others may be protected and saved.

Economic conditions have changed so much that many of the long-established medical and surgical procedures which were applied without question to individual animals are justified no longer for financial reasons. On the other hand, the advances that have been made in the knowledge of the natural history

(The March issue of The British Veterinary Journal was published on March 26)

of diseases, and especially of those incited by micro-organisms, has led to a better appreciation of the nature and value of prophylactic, eliminative, and specific curative methods of therapy.

In the past many individual animals were submitted to periods of treatment which might last for six or seven weeks. If resolution was effected ultimately the owners and their veterinary surgeons were satisfied. If the treatment failed then they would express regret but get solace from the fact that they had tried to effect a cure. Under present conditions the cost of feeding, the provision of adequate attention and the loss of earning power during the illness rules out any economic benefit that might accrue. In the case of commercial stock, be they on the farm or employed in transport, the modern veterinary surgeon must justify his existence according to sound economic standards. If he can prevent the occurrence of disease his services are of much greater value to a client than any prowess in effecting resolution after the damage has been done.

The pursuance of this aim has changed the face of veterinary science. Sound advice and good judgment can earn more dividends for client and surgeon than the knife or the bottle. For instance, in many cases of infertility the best course to follow would be to curb one's urge to attempt a cure and tell the client that, in his own interests and also in the interest of the herd in the present and in the future, the best policy would be to call in a butcher.

Our visitor from the past would be amazed at the high state of knowledge concerning the nutritional requirements of the animal body. Gone are the days when the fullness of the manger was the standard by which those needs were set. In the old days many dairy cows cost more to feed than they returned in the bucket and the owner remained solvent only because a number of his beasts were natural physiological economic units and were able to carry the others on their backs. Working and pleasure horses consumed quantities of grain far beyond their requirements. Good nutrition is the gateway to health and the services of a veterinary surgeon are most valuable on those occasions which his sound advice can ensure that he has no disease to treat. These fundamental changes in the art of veterinary practice do not imply that the diseases and abnormalities of the individual are of no further consequence. It is by the study of the individual that we can get a clear picture of the ætiology and pathogenesis of disease. Students must still sit beside the patient and get to know him and his troubles so that they may cultivate and enhance their powers of observation and deduction and employ the acquired talent with confidence and competence on a wider biological scale.

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GENERAL ARTICLES

ENCEPHALITIS IN CANINE DISTEMPER

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Introduction

IT has been recognised for a long time that encephalitis occurs quite often in the dog as one of the generalised effects of distemper infection. This fact was recorded by Pierret (1882) and by Matsulevich (1884) and since that time numerous papers have appeared which reflect undiminished interest, though by no means continuous agreement, on the causes and pattern of canine encephalitis.

Several early papers dealt mainly with lesions in the spinal cord (Pierret, 1882; Wood, 1885; Triboulet, 1893; Carougeau, 1898; Taty and Jaquin, 1898; Marchand and Petit, 1912*a* and *c*). Dexler (1904) described his neuropathological findings in three adult dogs which had previously suffered from distemper and, as in the cases of encephalitis of adult animals recorded by Batten and Holmes (1908), Batten (1913) and Flexner and Clark (1913), perivascular lymphocytic infiltration was a prominent lesion. This phenomenon also featured in cases studied by Cerletti (1912) and McGowan and Dawson (1914). In a series of 15 dogs with "nervous distemper" examined by Kantorowicz and Lewy (1923) five showed protozan parasites associated with encephalitis which the authors were prompted to suggest might be the causal agent. Goldberg and Volgenau (1925) described hyperæmia, hæmorrhage, perivascular lymphocytic "cuffing," chromatolysis and vacuolation of myelin in the cerebellum and pons. Encephalomyelitis with low-grade meningitis occurred in three out of thirteen distemper cases observed by Roman and Lapp (1925).

The experimental work of Dunkin and Laidlaw (1926) was concerned mainly with distemper virus, its transmissibility and associated serological and immunological phenomena. Some cellular reaction in the brain was briefly noted but changes occurring in this acute encephalitis were not considered characteristic for the disease. Gallego (1928) was unable to distinguish a typical histological picture for nervous distemper owing to variation in the lesions found in 20 dogs but he gave a detailed analysis of his findings and subdivided them under four headings, viz., vascular changes, modifications of the microglia, disturbances of the neuroglia (astrocytes) and lesions of the neurones. Perdrau and Pugh (1930) described encephalomyelitis in dogs, some of which had previously shown distemper signs. Perivascular lymphocytic infiltration was the principal lesion found but four out of 14 animals also showed demyelination,

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chiefly in the cerebellum and brain stem. Most of the dogs in this series were over one year in age and had been clinically ill for more than a month. Protozoa resembling those encountered by Kantorowicz and Lewy were seen in one animal. Posrednik (1930) examined 15 experimental and naturally occurring cases of distemper. The latter showed demyelinating lesions as well as perivascular "cuffing." Inclusion bodies were not found. Seifried (1931) drew attention to similarities between the encephalitis of swine fever and canine distemper, especially in the distribution of lesions in the basal ganglia, cerebellum, brain stem and paraventricular areas. According to Marinesco, Dragenesco and Stroesco (1933) the nervous changes in distemper were those of a disseminated encephalomyelitis characterised mainly by lymphocytic infiltration around vessels. Acidophilic inclusion bodies, usually nuclear in situation, were found also. Frauchiger and Walthard (1935) were unable to draw up a uniform histological picture of the encephalitis and encephalomyelitis encountered in seven cases of "nervous" distemper; nor did they recognise inclusion bodies specific for the disease. Peters and Yamagiwa (1936) examined the central nervous system in 16 cases of "nervous" distemper which they subdivided into two groups, the first of these being characterised by perivascular infiltrations of lymphocytes and plasma cells; the second and larger group showing in addition, vascular "proliferation" involving mainly the cerebral cortex, and focal gliosis concentrated in the white matter of the cerebellar folia without specific localisation near vessels. Parasites resembling those described by Kantorowicz and Lewy were also found in a few cases. De Monbreun (1937) observed meningitis, cerebellar gliosis and neuronal regressive changes in the cerebrum in certain of his cases of natural distemper. Cytoplasmic and intranuclear inclusions occurred in the glia. The experimental cases described in the same paper appear to have been complicated by infection with contagious hepatitis virus from the dog of group III in the series as the macroscopic and histological findings conform largely to those of Rubarth (1947). A disseminated encephalomyelitis involving perivascular infiltrations and demyelination (the latter in the cerebellum especially) was described in a four-year-old pointer by King (1939). Encephalitis in natural and experimental distemper studied by Cordy (1942) varied from slight inflammation with regressive cellular changes to extensive gliosis and degeneration, including demyelination which was mainly present in the cerebellum and brain stem. Disseminated encephalomyelitis with severe perivascular "cuffing" was found in 13 dogs, all of which were mature animals. Nuclear inclusions were recognised in the glia in nine out of 19 dogs with clinical nervous distemper and in the neurones in five of the cases with disseminated lesions. Cytoplasmic inclusions were occasionally seen in the ependyma and glia of experimental dogs only. In contrast with most of the previous authors, Hurst, Cooke and Melvin (1943) found very uniform changes in nine dogs suffering from "nervous" distemper, particularly in the distribution of lesions in the cerebellum, brain stem and surface of the interpeduncular fossa. The virus itself was held responsible for the demyelination present, and intranuclear glial and

ependymal inclusions were seen. Scherer (1944) described various forms of canine encephalitis, including a diffuse "proliferation" of cortical vessels, a multiple "sclerosis" with focal demyelination, and a disseminated perivascular encephalitis of chronic type. In each case he was doubtful of a connection with distemper notwithstanding the claims of previous writers.

A radical attempt to clarify the problem was made by McIntyre, Trevan and Montgomerie (1948) who subdivided canine encephalitis into five main groups. Their classification included a non-inflammatory disorder resembling that described by Dunkin and Laidlaw (Group Ia); demyelinating forms of encephalitis (Groups Ib and II); a perivascular lymphocytic lesion (Group III); toxoplasmosis (Group IV), and conditions attributed to nutritional or toxic factors. Though later modified to some extent by Trevan (1953) the original work served as a stimulus to a number of workers in Europe, Australia and the United States, who described aspects of the pathology of distemper, including those lesions which arose in the brain and spinal cord (Innes, 1949; Koprowski, *et al.*, 1950; Whittem and Blood, 1950; Winqvist, 1950). A valuable clinical and pathological account was given by McGovern, Steel, Wyke and Dodson (1950) who attributed a tremor syndrome ("chorea") in dogs to a meningo-encephalomyelitis characterised in its acute form by focal demyelination concentrated in the cerebellum and neuronal degeneration in the motor and premotor cortex, but which, in chronic cases, showed thickening of vascular walls, lymphocytic cuffing and degeneration of pyramidal cells of the cerebral cortex. Intranuclear glial inclusions were found. Focal demyelination was prominent among the findings of Cohrs (1951), Scheitlin, Seiferle and Stunzi (1951) and Fankhauser (1951). Cohrs (1951) regarded as doubtful the ætiological unity of those forms of canine encephalitis which he described, viz., a lymphocytic and plasma cell perivascular reaction, a focal "proliferation" of vessels and a demyelinating encephalitis. He also postulated the occurrence of an atypical distemper or some unrelated virus, whereas Fankhauser regarded the problem of viral plurality as still unsolved.

In a study of experimentally infected dogs, Röhrer (1951) found reactive changes by vascular endothelial and adventitial cells, and regressive neuronal signs especially in the hippocampus and cerebellar cortex. Potel (1951, 1954) concluded from his extensive observations on the pathology of experimental and natural distemper that the neurological changes consisted mainly of ischæmic regressive changes in the neurones; leptomeningitis, situated especially in basilar areas; swelling of vascular walls and their adventitial cells; and sometimes gliosis in periventricular areas of the posterior brain. Most of his cases were examined in the earlier stages of the infection, i.e., before the fifth week. Ribelin (1953) regarded 43 of his 56 cases of canine encephalitis as due to distemper infection because inclusion bodies (nuclear and cytoplasmic) were present or because ferret inoculation or a serum neutralisation test proved positive. The lesions, which most consistently affected the brain stem and cerebellum, showed perivascular reactions, microglial rod-forms and acute neuronal changes in less than half of these cases. By repeated passage of virus from a case of non-nervous distemper,

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Gillespie and Rickard (1956) produced experimental encephalitis in dogs, the intracerebral route of infection proving most successful. In 31 brains examined the cerebellum showed leptomeningitis, perivascular cuffing, and neuronal degeneration and myelin degeneration. Intranuclear inclusions were sometimes found.

Present Investigation

The 50 animals described in the present series have been the subject of a general clinical and pathological study by Lauder, *et al.* (1954). They comprised natural cases of infection examined at out-patient clinics of the University Veterinary School, diagnosed clinically as distemper and ultimately brought to post-mortem examination. Most of the dogs were killed by electrocution or by a barbiturate. Usually the autopsy was carried out within six hours of death but in a few cases a period of about 24 hours elapsed before the selected tissues could be placed in fixative.

An examination of brain tissue was made in all cases. The spinal cord was examined in 25 dogs, by means of transverse sections taken from the anterior and posterior thirds of the cervical, thoracic and lumbar areas.

Methods

In most cases portions of brain were selected for histological examination as soon as the dura mater was removed at autopsy. Central cerebral cortex, cerebellum and pons were examined in almost every dog, usually being supplemented by parietal cortex, basal ganglia and thalamus. Other areas studied especially in the latter part of the survey were olfactory tract, frontal cortex, hippocampus, occipital and temporal cortex, mid-brain and medulla. An examination of brains by a series of transverse sections was adopted only in the last few cases of the survey but has since been used almost exclusively in a later series of encephalitic dogs not included in this paper.

Tissues were fixed in 10 per cent formal-saline except for some later cases in which formal-sublimate was used. Occasionally, portions of cerebrum and cerebellum were fixed in formal-ammonium bromide prior to gold impregnation.

Except for tissues prepared as frozen sections, all blocks were embedded in paraffin wax.

The hæmalum-phloxin-tartrazine method of Lendrum was used as a general stain which also proved invaluable for the demonstration of cytoplasmic inclusion bodies. Additional methods employed from time to time were Loyez' hæmatoxylin, hæmalum-eosin, phosphotungstic acid-hæmatoxylin, Gram-Twort, Van Gieson, Gordon and Sweets' silver method, and the Feulgen and periodic acid-Schiff histochemical techniques. Cajal's gold-sublimate and Bielschowsky's methods were used for the demonstration of astrocytes and axons respectively.

Morbid Anatomy

The most consistent change was congestion of the meningeal and deep vessels of the brain and those of the choroid plexuses. The degree of hyperæmia

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varied greatly; in the severer cases it was not unusual to find the cerebro-spinal fluid somewhat opaque and even tinged with blood. There was often an increase of cerebro-spinal fluid in extreme cases of sufficient degree to distend the ventricles. Especially in relation to the choroid plexus systems and the leptomeninges close to the optic chiasma, a turbid, semi-opaque greyish fluid could frequently be seen in the subarachnoid space. The most seriously inflamed brains often appeared œdematous.

Histopathology

Only one out of the series of 50 dogs failed to show encephalitis histologically. While it is possible that no inflammatory reaction was present in the brain or cord, or that resolution had occurred, it may be that the examination, limited to only four blocks of brain tissue, was responsible for the negative findings in that case. Clinically, the animal had shown chorea in both hind limbs, and histological changes in the lungs and pads suggested an advanced stage of distemper.

Since the dogs in this series were naturally occurring cases of distemper, most of which had ailed clinically for three weeks or longer, the histological changes detailed in the central nervous system, as elsewhere, do not necessarily represent the initial tissue response to virus infection. Some of the changes seen in dogs with the longer clinical histories were obviously of quite extended duration. Despite the pitfalls in assessing the approximate length of illness in natural cases of distemper (see Lauder, *et al.*, 1954), the findings in the present series suggest that the neuropathological changes, while variable from animal to animal, nevertheless have a fairly constant pattern of cytological character, distribution and sequence of development. These characteristics are described in the following paragraphs.

The Neuroglia.

(*a*) *Microglia:* Morphological changes in the microglia were among the earliest detectable lesions and offered a sensitive sign of tissue reaction. The subpial zones of the thalamus, mid-brain, pons and medulla were often the site of a microglial response, (Fig. 1) especially where dense myelinated bundles occurred, e.g., at the emergence of cranial nerves. A similar relationship of myelinated tissue was often noted in the deeper medullary tissue of the cerebellar folia and at those points where the pia mater was directly applied to superficial white matter of the cerebellum (Figs. 2, 2*a*) and to the tissue contiguous with the recessess of the fourth ventricle; microglial reactions occurred to a lesser degree in deeper areas, although they developed readily near acute vascular reactions.

The earliest morphological changes in the mesenchymal glia were hypertrophy and alteration in shape. The nucleus assumed an elongated, sometimes twisted or ribbon-like form (" rod cells ") followed by an increase in cytoplasmic volume whereby the cell showed clearly an ovoid, rounded or irregular shape. Mitotic division was sometimes seen. At this stage, early astrocytosis and

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oligodendroglial swelling had frequently developed as well (Fig. 3) and eventually there were distinct foci of gliosis. With the development of myelinoclasis, many microglia become greatly enlarged, with a granular or foamy cytoplasm (" lattice cells ") which often reacted positively to periodic acid-Schiff staining (Fig. 4). The number of foamy cells varied directly according to the degree of tissue breakdown. In frankly necrotic areas of the brain and cord, the microglia were abundant as lattice (" gitter ") cells. Phagocytosis of cellular fragments was in evidence. Microglia in all reactive stages often contained phloxinophilic cytoplasmic inclusions. Degenerative microglia were common in the severe lesions. A fall in the concentration of microglia but persistence of hypertrophic astrocytes in groups was a feature of some cases, which probably denoted a late encephalitic stage (Fig. 6). (Scarring: *vide infra.*)

(*b*) *Astrocytes:* In the earlier phases of reaction, i.e., in most of the dogs with the shorter histories of illness, the astrocytic response ran more or less parallel with microgliosis. Swelling of cytoplasm and nucleus rendered the cells abnormally conspicuous, while at the same time they lost processes (Fig. 5*a*). In myelinoclastic or frankly necrotic lesions an increase of astrocytes sometimes appeared to occur. Plump (" gemastete ") astrocytes were usually a feature of the demyelinating area, when the cells stood out clearly, even, for example, in hæmalum and eosin preparations, because of their pale, basophilic, hyaline cytoplasm. Variation was found both in the size and shape of reactive astrocytes. Multinucleated forms were not uncommon and giant astrocytes with bizarre nuclei developed occasionally. In some cases (eight out of 50), groups of hypertrophic cells recognisable less by their cytoplasm, which was relatively inconspicuous, than by their enlarged nuclei, could be identified in the myelinated zones of the cerebellum or elsewhere when less alteration was apparent in either of the other glial types (Fig. 6). Such lesions may have represented a late healing stage and, indeed, occurred in dogs with lesion-patterns suggestive of a late stage in the disease. (Six of the eight dogs so affected had clinical illnesses of between four to eight weeks.)

In acute lesions it was usual to recognise phloxinophilic inclusion bodies in the cytoplasm of astrocytes, as in the other glia, and these occurred both singly and in groups (Fig. 7). Astrocytes were notable, however, in commonly showing large intranuclear bodies associated with margination of chromatin (Fig. 8). Such structures were acidophilic; though not unlike the intranuclear inclusions of canine contagious hepatitis morphologically, they differed from the latter in being Feulgen-negative. A number of authors have described these " inclusion-bodies " as the only form to be found in distemper.

(*c*) *Oligodendrocytes:* Acute hydropic swelling of oligoglia, localised in distribution, was a frequent change and corresponded to the distribution of these cells in that it predominated in richly myelinated zones (Fig. 3). Thus the encephalitic lesions in the cerebellar folia and cranial nerve roots of the brain stem usually showed numbers of large round cells with colourless cytoplasm and compressed peripheral (" signet-ring ") nuclei. Their characteristic appearance acted as a useful landmark of the developing lesion since they were seen clearly

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even on low-power examination (Fig. 2). With the onset of demyelination the accumulation of many round colourless structures created a cribriform or "moth-eaten" pattern (Figs. 9 and 10: status cribrosus, status spongiosus, etat crible). At this stage it was not possible to relate every space with certainty to individual oligodendroglia. Other spaces showed a cylindroidal outline within which an axon could often be seen in a more or less central position. Oligodendrocytic nuclei were sometimes situated at the margins of these spaces, but the impression given was of a lesion affecting nerve fibre, and in particular its myelin sheath, together with the attendant neuroglia, rather than of a purely oligodendroglial change (Fig. 11).

As in the case of the other glial forms, periodic acid-Schiff positive granular or diffuse material sometimes occurred in the cytoplasm, especially where myelinoclasis was in progress. Cytoplasmic, phloxinophilic inclusion bodies also developed in oligodendrocytes. In the acute lesions nuclear breakdown could often be recognised.

Apart from the areas stated above, oligodendroglial change was elsewhere less marked, except in a few cases showing severe oligodendroglial swelling in the subcortical white matter of the cerebrum (Fig. 12). Vacuolated or atrophic neurones sometimes had groups of swollen satellite oligoglia, but comparatively little change was found in them in the vicinity of vascular lesions, except for those of more acute type. Late glial lesions containing clusters of hypertrophic astrocytes and sometimes microgliosis displayed few, if any, swollen oligodendrocytes when myelinoclasis was not present.

(*d*) *Ependyma:* In the acute phases of encephalitis, cytoplasmic inclusions could usually be found in the ependymal lining and paræpendymal cells (Fig. 13), particularly those of the third and fourth ventricles and mesencephalic canal. Reactive changes by subependymal astrocytes and microglia, such as cytoplasmic and nuclear hypertrophy, were seen frequently at the same time. Nuclear inclusions had developed in subependymal cells in a few cases. In the roof and lateral recesses of the fourth ventricle oligodendroglial swelling often occurred nearby.

Neurones (Excluding Fibres): Some degree of neuronal degeneration could be found in all cases, chromatolysis being foremost of these, but many cellular signs had to be regarded as impossible to interpret with certainty as ante-mortem. Euthanasia by chemical or electrical methods probably added to the morphological alterations in nerve cells and the delay which separated death of the animals from tissue fixation was yet another complication. It seems justifiable to assume that much of the change in neurones closely associated with inflammatory changes was of a pathological nature. Occasionally a neurone or group of cells showed degeneration far in advance of their neighbours, even in the absence of marked inflammation, and this circumstance also may well have been significant; isolated neuronal necrosis, a phenomenon infrequently seen, is an extreme example.

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Chromatolysis was, of all neuronal abnormalities, the most commonly encountered in both brain and spinal cord. It was often of a patchy nature, sometimes involving an isolated cell or two among many other neurones of normal appearance. The pathological significance of chromatolysis was usually impossible to determine with any assurance.

Cytoplasmic droplet formation or vacuolation occurred frequently, a positive periodic acid-Schiff reaction being given by the droplets in some cases. Neurones situated close to glial reaction or to vascular inflammation (especially acute forms) tended to show cytoplasmic changes and hypertrophy. Sometimes large groups of neurones, e.g., in the hippocampus, were greatly swollen. Nuclear abnormalities, including enlargement, nucleolar swelling and lytic change were seen also. Karyorrhexis was rare except in the neurones of the granular layer when lesions of the cerebellar folia impinged upon them.

Advanced cellular degeneration and necrosis occurred in neurones caught within the boundaries of intense glial reactions, or lying in the vicinity of acute vascular inflammation (see below). Extensive softening, allied to less acute perivascular infiltration, and involving death of neurones, may have represented an ischæmic process. This form of lesion occurred in one out of 25 spinal cords examined. In advanced lesions the cytoplasmic and nuclear changes mentioned could often be recognised. The onset of necrotic change was generally assumed to be overtaking neurones which showed extreme shrinkage, and lysis of cytoplasm or nucleus as suggested by marked loss of tinctorial affinity and cellular detail. Satellitism by swollen oligodendrocytes and other glia was then common.

In a few dogs, nerve cells in the grey substance of the spinal cord appeared to be encrusted by deeply basophilic granules (" boutons terminaux ").

Phloxinophilic inclusion bodies sometimes developed in the cytoplasm of neurones. This was particularly so when these cells lay near or within areas of glial inflammation, but, in a few dogs with early acute encephalitis, inclusions occurred frequently in neurones throughout the brain although glial aggregations were as yet little developed.

Nerve Fibres: Degeneration of myelin was found in 25 cases, most commonly in the principal areas of glial reaction previously described, viz., the white substance of the cerebellar folia, deep cerebellar nerve roots; it also occurred in the subpial zones of the brain stem, cerebellar recesses, thalamus and corpus callosum. The superficial myelin of the brain seemed therefore most susceptible to damage. In general the degree of degeneration (myelinoclasis) ran parallel to the intensity of gliosis, and since in the early stages of reaction the glial changes seemed to precede myelinoclasis, they might, therefore, be considered a factor in its causation. The presence of foamy microglia containing periodic acid-Schiff positive material in mature demyelinating lesions suggested that the subsequent liberation of breakdown products from the fibres (e.g., glycolipid) may intensify the glial response (Fig. 4).

Degradation of myelin was shown first by uniform or corded swelling and by vacuolation (Fig. 11), although the latter could not be related always with

certainty to myelin. Clear ovoid or rounded spaces of different sizes were encountered in the white matter. The smaller sometimes seemed to be within the confines of the myelin, but equally many of the larger spaces might have existed between fibres, or have represented hydropic oligodendrocytes or œdema fluid. Thus a series of vacuoles or spaces in myelin, oligodendrocytes and possibly interfibrillar areas were so closely involved as to make not only their localisation but also their interpretation a matter of great difficulty. The degree of vacuolation, like that of the gliosis, varied more or less directly with the amount of myelin damage.

With the accumulation of neuroglia the myelin disappeared progressively until none might be identified in, for example, a section of an inflamed cerebellar lamella (Fig. 10). Silver impregnations were seldom carried out in the present study, but it is thought that axons can sometimes persist intact after the removal of their myelin.

In large areas of softening associated with dense perivascular infiltration, which have occurred in the brain or cord of several cases, massive breakdown of fibre architecture coincided with dense glial hyperplasia and infiltration. Lattice cell macrophages then predominated.

Blood Vessels: It was a feature of this series that the severity of structural change in vascular walls and perivascular zones was generally greatest in dogs with illnesses of longer duration. Table I shows the incidence of acute vascular inflammation ("capillaritis") and of lymphocytic perivascular infiltration (a more extended process) in relation to the length of clinical illness.

TABLE I

Duration of Illness	*Acute Changes*	*Perivascular Infiltration*
3 weeks	1/6 (16.6 per cent)	2/6 (33 per cent)
3 to 6 weeks	4/24 (16.6 per cent)	13/24 (54.2 per cent)
6 weeks	4/12 (33 per cent)	9/12 (75 per cent)
Indefinite	3/8 (37.5 per cent)	4/8 (50 per cent)

In addition to hyperæmia, the earliest vascular changes comprised swelling of capillary endothelium and thickening of the elements in arteriolar walls due possibly to an active contraction. Sometimes a slight measure of swelling or even proliferation was shown by adventitial cells (pericytes), but often this was not marked until later. Such lymphoid perivascular infiltration as was seen in the cases of Group I above was of low grade and very localised in distribution. Little histological change was evident in the structure of vessels even when situated close to young glial lesions. With the development of more advanced gliosis, capillaries or larger vessels incorporated in the inflammatory area tended to show hyperæmia, swelling of mural elements (including adventitial cells, sometimes with hyperplasia on their part) and the accumulation of lymphocytes, both intra- and extra-vascular, but usually these changes were overshadowed

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by the complex adaptations of glia and by the degradation of myelin, and seemed, therefore, to be secondary phenomena.

A severe but localised inflammation of small vessels (" capillaritis," " productive vascular foci "), apparently of primary type, was found in 12 dogs (24 per cent), eight of which had been clinically ill for three weeks or more. The cerebral cortex and cerebellar white matter were each involved in seven cases, the lesion being detected exclusively in the cerebrum in three of these. Especially in the affected sectors of cerebral cortex the lesions stood out prominently, even under low-power magnification, because of the swollen endothelium (Fig. 14). Some previous workers have considered the process an active capillary hyperplasia (Cerletti, 1912; Kantorowicz and Lewy, 1923; Cordy, 1942; Scherer, 1944; Innes, 1949; Scheitlin *et al.*, 1951; Cohrs, 1951), but this does not seem to be the case for, although division of endothelial cells is found occasionally, no obvious " budding " of vessels takes place. This view has been expressed previously by Koprowski *et al.* (1950), Fankhauser (1951) and Lauder *et al.* (1954). There is swelling and hyperplasia of adventitial and endothelial cells (Fig. 15), a light lymphocytic infiltration of perivascular spaces may occur, and the adjacent glia are in an intensely reactive state of swelling, hyperplasia (of microglia) and even acute disintegration. Regressive changes may develop in nearby neurones. Cytoplasmic inclusion bodies may be present, while astrocytes sometimes show intranuclear structures.

Perivascular " cuffing " occurred in 28 dogs (56 per cent) but was disseminated in only seven of these. When the process was advanced the Virchow-Robin space encircling the affected vessel became distended and packed by mononuclear cells, among which lymphocytes almost always predominated (Fig. 16). Plasma cells were sometimes seen but macrophages seldom took part in the infiltration. It was not uncommon to find a small proportion of the reactive cells invading the nervous parenchyma for a short distance. Compared with the acute vascular lesions, little change took place in the neighbouring glia except in cases complicated by massive softening of nervous tissues when usually the degree of " cuffing " was severe (Fig. 17); it seems possible that such degeneration denoted infarction. It was also found that inclusion bodies of both kinds were related generally to an earlier phase of reaction. Lymphocytic " cuffing " often developed around meningeal as well as deep vessels (Fig. 18). In both instances, veins were affected to a greater degree than arteries.

Often the perivascular spaces of Virchow-Robin which communicate with the subarachnoid channel were dilated, corresponding to the excess of cerebrospinal fluid usually to be seen even on macroscopic examination. Masses of metachromatic non-cellular material giving a positive periodic-acid Schiff reaction were present occasionally in the perivascular spaces, along which they gravitated apparently from deeper inflammatory lesions to the subarachnoid area. A similar fluid was found in the parenchyma of twelve brains of the series especially in damaged fields of myelin (Fig. 12).

Petechiation sometimes occurred in acute encephalitic areas, both glial and

vascular, but massive hæmorrhage was absent from the brains and cords of this series. Thrombosis could never be demonstrated convincingly.

The vessels of the choroid plexus systems were hyperæmic in many cases and their stroma quite often œdematous. Cytoplasmic inclusions rarely developed in the epithelium.

Meninges: Meningitis was usually present, although not necessarily severe or widespread. The ventral pia-arachnoid of the optic chiasma and its environs offered the most consistent opportunity of studying superficial changes. Swelling and desquamation of meningeal cells were the earliest indications of reaction and at that stage cytoplasmic inclusions could often be detected in them. Further development of meningitis showed an increase of free, round or oval mononuclears, increasing activity on the part of perivascular adventitial cells, as in deeper sites, and the progressive accumulation of hæmic elements, particularly lymphocytes (Fig. 18). Plasma cells were sometimes formed, but polymorphonuclears seldom appeared; secondary bacterial meningitis did not occur in this survey and the condition has been found rarely in an extended series of over 250 dogs with distemper infection. Encephalitis of longer duration characterised by perivascular infiltration always showed a similar meningitis which tended to develop widely over brain and spinal cord and the "cuffing" could usually be recognised as a continuous process in sections containing junctions from which vessels penetrated sulci in the nervous tissue.

Inclusion bodies: Cellular inclusion bodies have been found by most workers investigating canine encephalitis. Innes (1949) wrote of cytoplasmic inclusions. Both intracytoplasmic and intranuclear structures were described by Marinesco *et al.* (1933), Cordy (1942), Hurst (1943), Sjolte (1947), McIntyre *et al.* (1948), Winqvist (1950), Whittem and Blood (1950), Scheitlin *et al.* (1951), and Gillespie and Rickard (1956). In the present series, cytoplasmic bodies were the most prominent form (Lauder *et al.*, 1954) but nuclear inclusions of an apparently distinct nature also occurred in 13 cases; because of their distinctive characters these will be described separately.

(*a*) *Cytoplasmic inclusions:* These were identified in the central nervous system in 34 dogs (68 per cent), especially in the brain. The relative concentration of inclusions in other organs has been stated by Lauder *et al.* (1954). In the brain their development usually ran parallel to the distribution and severity of the glial inflammation in the primary phase of encephalitis. Among the areas in which they could be detected most readily in the earlier stages of reaction were the subpial and ependymal layers of the thalamus, mid-brain and brain-stem. Zones of gliosis in the paraventricular tissue of the fourth ventricle and in the cerebellar white matter were useful for the detection of inclusions, as were the meninges where fixed and free macrophages commonly contained them. The spinal cord invariably showed fewer inclusions than the brain. In one three-month-old terrier which had been clinically ill for 14 days many of the acutely swollen glia throughout the brain showed inclusions; rather fewer occurred in neurones, perivascular adventitial cells and meninges. Such widely distributed

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and high concentration of cytoplasmic inclusions was exceptional, occurring in extreme form in this case which was one of the youngest dogs in the series, and in a poodle of about three years, one of the oldest.

Cytoplasmic bodies developed in all of the glial forms (*cf.* intranuclear inclusions), but particularly in the microglia and astrocytes. When glial disintegration took place they were apparently capable of existing freely for some time among the tissue debris.

The morphology of these inclusions has been described by Lentz (1907), Standfuss (1908), Babes and Starcovici (1912), Sanfelice (1915), Nicolau (1935) and others. In the present work they stained strongly with phloxin and could be differentiated with ease from cellular cytoplasm. No inclusion of this type was conclusively identified in a cell nucleus, although claims have been made for such localisation (Sjolte, 1947). From a comparative study of case histories and the development of lesions throughout the body it appears probable that apart from misinterpretations brought about by the use of less critical staining methods, such as hæmatoxylin-eosin, failure to demonstrate inclusion bodies in the brain (as elsewhere) is largely governed by the stage of the disease process (Lauder *et al.*, 1954). Under "field" conditions of study the absence of inclusions from the central nervous system seems most likely to be associated with the prolonged case having a history of six weeks' illness or more. This provisional estimate of the persistence of cytoplasmic inclusions is made from the examination of an extended series of over 250 dogs, which also provided some evidence that they may persist in the brain after they have disappeared to a great extent from the viscera, with the possible exception of the lung.

(*b*) *Intranuclear inclusions:* These were, to a great extent, a feature of the astrocytes, but they sometimes occurred in oligodendrocytes and the ependymal lining, both of these being related elements. Cerebellar and pontine areas of gliosis which included proliferations of astrocytes often showed them in greatest numbers (Fig. 8). They also developed elsewhere in the brain, e.g., cortical zones of acute reaction in the cerebrum, and in the cord. Their main tinctorial reactions, after formol-saline or formol-corrosive fixation, were a dull basophilia by hæmalum-phloxin-tartrazine and periodic acid-Schiff, eosinophilia by hæmalum-eosin and the common hæmatological stains and a negative Feulgen reaction. In the latter respect they differed from the other common intranuclear inclusion body of canine virus disease seen in contagious hepatitis (Rubarth, 1947), which is Feulgen-positive.

As the distemper-type inclusion developed as a round, ovoid, rhomboidal or irregular mass, the nuclear chromatin dispersed into numerous small particles until it was concentrated at the periphery ("margination"). Though formed alongside the nucleolus in the earlier stages only the fully developed inclusion, occupying the greater part of the nucleus, could be seen ultimately. Nuclei contained several of them occasionally. The nuclei of multinucleated astrocytes also developed inclusions. A small proportion of cells showed both nuclear and cytoplasmic bodies.

Toxoplasmosis. Two of the 50 dogs showed toxoplasma infection of the brain (Fig. 19). Organisms were not identified in the central nervous system of a third case with an infected bronchial lymph node. Both positive animals showed an extremely localised form, one consisting of several "pseudocysts" which had elicited a focal gliosis in the frontal cortex of the cerebrum, the other an equally small lesion in the substantia nigra of the mid-brain. The protozoan infection in all three animals constituted a subclinical process which may have been non-progressive, prior to the onset of distemper. The significance of latent toxoplasmosis in the dog was discussed by Campbell *et al.* (1955).

Discussion

The encephalitic forms described, which occurred during natural distemper infection of city dogs, correspond essentially to the pattern found in a larger series of cases compiled later and at present under scrutiny. It is noteworthy that the changes previously studied under experimental conditions by Dunkin and Laidlaw (1926), Cordy (1942), McIntyre *et al.* (1948), Röhrer (1951) and Potel (1951, 1954) have been generally less severe than those recorded in natural cases, including the present series. This apparent discrepancy might be explained by variation in the neurotropism of strains of virus (although the degree of pathological change is not necessarily an index on the concentration of virus [see McIntyre, *et al.*, 1949]). On the other hand it seems likely, as an examination of the papers mentioned will show, that many experimental dogs have been sacrificed at a stage of infection beyond which the spontaneous case often survives. In other words, those who studied experimental distemper have concentrated on the earlier stages of the disease, while accounts of natural distemper frequently relate to a process which has been allowed to mature through the passage of time though, perhaps, only a few weeks. In the series of 59 experimental dogs studied by Potel (1951), 47 were killed before the fifth week after infection, and it was noted that the disease had progressed for a "relatively long period" in a number of cases showing encephalitic lesions of the cerebellum, pons and medulla. It may be significant also that the neurotropism of distemper virus seems to be enhanced by serial passage, as described by Gillespie and Rickard (1956), who reduced the period for the development of clinical nervous signs to 6 to 16 days. Inflammation, in the form of gliosis, was most marked in those dogs developing nervous manifestations 22 or more days after inoculation, the intracerebral route being used. Data obtained in the present series strongly suggest that the neuropathological picture varies widely even over a period of a few weeks; for example, that the brain lesions after a three-week infection contrast greatly with a process of six weeks' standing.

Nevertheless, the pathology appeared to follow an extremely regular sequence of development and pattern of distribution.

Among the earliest cellular changes seen was activation of microglia and astrocytes. Generally this could be found most easily in the zone lying immediately beneath the pia of the pons, cerebellar recesses and superficial white matter, mid-brain, lateral and ventral thalamus, chiasma and corpus callosum. It also

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occurred subependymally. Together with macrophage activity in the meninges, therefore, it showed the qualities of a surface reaction and suggested in this anatomical respect a possible relationship to cerebrospinal fluid as a carrier of virus. (See also Marinesco *et al.*, 1933; Potel, 1951, 1954). The development of cytoplasmic inclusion bodies in the reactive cells again pointed to the virus itself as being directly related to this encephalitic stage. More advanced lesions were often found in the posterior cranial nerve roots, deep cerebellar white matter of the folia, especially where it impinged on the pia mater, and in the paraventricular tissue of the fourth ventricle.

Mature lesions showed two main tissue changes, the glial alterations and some degree of nerve fibre degeneration. From the histological viewpoint, glial swelling, proliferation and even disintegration appeared primary, and myelinoclasis secondary, but the exact sequence and pathogenesis can only be surmised. That virus proliferates within glia and leads in some way to the production of a factor or factors toxic to myelin seems possible. Demyelination is followed readily by an exaggerated gliosis in which foamy lattice-cell microglia (gitterzellen) are prominent, just as it is in areas of softening in nervous tissue generally. The presence in the cerebellum of groups of hypertrophic astrocytes with only a subdued reaction by other glia was seen in a few cases, mostly of comparatively long standing, and may have represented post-inflammatory scars, but it was not found possible to detect and so study such lesions fully in the limited number of Cajal gold-sublimate preparations prepared from this series.

Like the other neuroglia, oligodendrocytes responded sensitively to the lesions of white matter, so that acutely swollen cells were among the earliest abnormalities in the nerve roots and richly myelinated areas of the cerebellum and its penduncles. The possible role of oligodendroglia as nutritional satellites of myelin has been discussed by Lumsden (1951), and from the present study it may be suggested that their reaction reflects a disturbance of myelin function. Alterations in the structure of medullated sheaths, e.g., cording and vacuolation, were prominent in well-developed lesions. Frequently it was impossible to determine whether the colourless spaces observed represented hypertrophic oligodendrocytes or greatly swollen sheaths. It is conceivable from the purely microscopical aspect that coalescence of the two structures or their breakdown products can occur.

In the lesions which have been discussed so far, inflammation of blood vessels played a comparatively minor part. Swelling of vascular walls and reactive hypertrophy and hyperplasia of adventitial cells (pericytes) usually developed to a limited extent only in the earlier lesions in those vessels incorporated in areas of gliosis, and perivascular infiltrations were subdued. The early distemper encephalitis is, therefore, predominantly glial in type, and situated mainly in subpial, subependymal and myelinated areas of brain, especially in its posterior parts. The spinal cord suffered to a much more limited degree.

That is not to say vascular changes are uncommon in distemper. In almost every previous account of canine encephalitis, changes in and around blood-

vessels have figured quite largely and the present survey has likewise revealed, in many cases, alterations in the vascular bed of brain and cord concurrent with modifications in the glia. From a study of the clinical histories, however, the majority of those dogs with acute " capillaritis," perivascular infiltration or both changes had shown a longer illness, usually of between one and two months (Table I). Further, in a high proportion of such animals with a shorter clinical illness, the general histological picture shown by other tissues such as lymph nodes, spleen and lung suggested infection of longer duration; it may be that the clinical history is not always a true reflection of the duration of disease process (Lauder *et al.,* 1954).

Acute " capillaritis," one of the two main vascular lesions found, occurred in 12 dogs and was commonest in the cerebral cortex. The acuteness of this process was indicated by the reactive changes in the adventitial cells and adjacent glia, sometimes including mitosis, and by frequent evidence of their disintegration. Cytoplasmic inclusions were not always seen in or near areas of this kind, a fact which, in conjunction with their anatomical difference from the previous lesions discussed, provokes the hypothesis that they are not ætiologically identical. This question arises again from the perivascular infiltration, largely lymphocytic, observed in 28 dogs, and found with even less specific localisation at all levels of brain and cord. The appearance of the lymphoid reaction generally coincided with signs of generalised hyperplasia in the nodes, spleen, tonsillar and intestinal lymphoid reservoirs (Lauder *et al.,* 1954), so that the infiltration seemed to be part of a body-wide response. The delayed nature of the proliferation and the type of cell involved are not inconsistent with an immune reaction. The possibility of an allergic process has been put forward by Hurst *et al.* (1943), Fankhauser (1951), Hurst (1952) and Trevan (1953). In many reported cases of canine nervous disease characterised largely by a disseminated lymphocytic and perivascular meningoencephalomyelitis, the authors were unable to detect inclusion bodies (Frauchiger and Walthard, 1935; Peters and Yamagiwa, 1936; Scherer, 1944; Innes, 1949; Fankhauser, 1951); in some, transmission experiments to dogs or ferrets proved negative (McIntyre *et al.,* 1948; Innes, 1949). Both of these results may have been due in some measure to the late stage at which the disease was studied, when virus was no longer present, or much reduced in concentration. The animals in Group III of the encephalitides described by McIntyre *et al.* (1948) fall into this category.

From a comparative study of the cases in this series it is, therefore, considered that the duration of illness is a major factor in the histological variation in distemper encephalitis. Many experimental cases would be required to study the significance of the time factor. Already the work of Potel (1951, 1955) and Gillespie and Rickard (1956) has provided new knowledge on this aspect of canine encephalitis, especially in relation to the earlier stages of infection, but gaps in the understanding of the later phases of encephalomyelitis remain. Acute capillaritis and perivascular lymphocytic " cuffing " as described by several authors (see above) have proved outstanding features of the later cases for reasons still to be determined. Sporadic and even enzootic cases of disseminated peri-

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vascular encephalomyelitis have not always been associated by the clinical or laboratory workers with distemper infection. Not a few of these cases have developed in older dogs of 18 months or over and the present survey would seem to suggest that this affection may in some cases be a late or post-distemper development (see also McGovern, *et al.*, 1950, and Trevan, 1953).

The occurrence of two distinct forms of inclusion body was previously noted in the communications of Hurst, *et al.* (1943), Winqvist (1950), Cohrs (1951), Trevan (1953), Ribelin (1953) and by Lauder, *et al.* (1954) who discussed the general problem of their significance and demonstration. The cytoplasmic bodies are similar to those found in many other tissues throughout the body (Sjolte, 1947; Lauder, *et al.*, 1954). Moulton and Brown (1954) showed, by means of a fluorescent antibody technique, that they are closely related to the viral antigen itself.* Of the 50 cases examined in the present series, 44 showed cytoplasmic inclusions in some parts of the body; 34 exhibited inclusions to the central nervous system, particularly in the brain where reactive glia and meningeal cells were commonly involved. Nine dogs displayed inclusions in the brain although they were scanty in, or absent from, other tissues examined; of this group seven had been ill for periods of six to eight weeks and two for five weeks which gives the impression that inclusions may persist until a later stage of the disease in the nervous system.

The status of the nuclear "inclusion bodies" remains uncertain but it is clear that in their tissue affinity, intracellular locus and staining characters they differ from the cytoplasmic structures (Lauder, *et al.*, 1954). In the central nervous system they develop in astrocytes and sometimes in their related cells, the oligodendroglia and ependyma. Identical bodies have not yet been found by the present author in the absence of distemper infection and the disease appears to be unique in showing two such clearly defined yet, it would seem, morphologically distinct inclusion. Care must be taken in diagnostic histopathology to differentiate between the intranuclear inclusion bodies of distemper and those of canine contagious hepatitis (Rubarth, 1947) but the latter are of wider distribution, usually stain basophilic in hæmalum-eosin or Romanowsky preparations and are positive in reaction to Feulgen's method.

No clear correlation between clinical signs and nervous lesions was obtained from this investigation. Thirty-nine dogs showed obvious signs including the regional myoclonic spasms termed chorea (23 cases), epileptiform seizures (25 cases), degrees of posterior incoordination or paraplegia (eight cases) and hysteria (seven cases). Often, more than one of these were present. It is noteworthy that of 11 dogs without observed nervous signs, nine showed inflammation in the cerebello-pontine area, and five demyelination, three of them being severely affected.

* Since the preparation of this paper, Moulton has described the demonstration of distemper virus in nervous lesions of dogs by the fluorescent antibody technique (*Proc. Soc. exp. Biol. Med.* (1956) *91*, 460). The writer did not recognise cytoplasmic inclusions in ordinary stained preparations of brain. If this was due to the methods employed (see Lauder, *et al.*, 1954) it is still not clear whether the fluorescent structures demonstrated were cytoplasmic or nuclear in position.

A full understanding of the incidence of encephalitis in distemper has yet to be attained. Many previous studies have concentrated on cases of clinical "nervous" distemper (Kantorowicz and Lewy, 1923; Marinesco, *et al.*, 1933; Frauchiger and Walthard, 1935; Peters and Yamagiwa, 1936; Scherer, 1944; McGovern, *et al.*, 1950), which almost always showed inflammatory lesions in the brain on histological examination. Some workers investigating naturally occurring cases have included examples of "catarrhal" distemper, but the neuropathology of such dogs did not appear clearly different from that of animals with "chorea," epileptiform or other nervous signs (Cerletti, 1912; Lauder, *et al.*, 1954). Comparatively few authors described the pathology of experimental distemper. The account by Dunkin and Laidlaw (1926) is brief and like that of Potel (1951) may have included a large proportion of animals in comparatively early stages of infection (see Röhrer, 1951; Potel, 1954, 1955). In a series of 66 dogs clinically diagnosed as encephalitic, Ribelin (1953) regarded 56 as definitely related to, or following distemper; of the latter, 43 (93.4 per cent) showed inclusions in the bladder, adrenal, ependyma or neuroglia. Those described in the central nervous system (25 cases) were mainly intranuclear. The degree of tissue change varied from severe degeneration and inflammation to none (two dogs). The present group of 50 distemper cases showed, with one exception, degrees of encephalitis; cytoplasmic inclusions were detected in 34 (68 per cent). From these two surveys, therefore, the incidence of encephalitis in naturally occurring distemper would appear to be high, but until more extensive investigations are pursued into the pathology of the less severe cases of "street" distemper and of experimental distemper, a final assessment of the importance of inflammation in the neuraxis cannot be reached. The fact that three cases of severe demyelinating encephalitis in the present series were not observed to have nervous signs shows the fallacy of basing an estimate of encephalitis on clinical examination alone. (See also Singleton and Hunter, 1956).

As to the factors which may influence the severity of encephalomyelitis, little substantial evidence can be drawn from work carried out to date. Gillespie and Rickard (1956) showed that intracerebral passage may exalt the virulence of distemper virus. The interplay between a virus and the tissues of the host is complex and may well be influenced on the one hand by such factors as variation in viral strains, infective dose and route of inoculation; on the other by age, breed, plane of nutrition and immunity. Only through experimental studies by clinical, pathological and immunological methods can these factors be fully assessed.

Summary

A neuropathological study had been carried out on 50 dogs suffering from naturally acquired distemper. Encephalitis was detected in all except one of the series and was associated in 25 cases with demyelination. The cerebellum and brain stem were mainly involved, areas of superficially disposed myelin appearing most susceptible to damage. Vascular changes included an acute inflammation of small vessels, especially in the cerebral cortex, and a disseminated

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perivascular infiltration by lymphocytes. The latter occurred most commonly in cases of longer duration. Two distinct forms of inclusion body are described.

Acknowledgments

I am indebted to colleagues in the Department of Veterinary Pathology and Medicine for help in assembling the material and for advice in its presentation; to Mrs. E. L. Leighton who assisted greatly in translations; to Messrs. W. Penny, F.I.M.L.T., and W. McLennan, F.I.M.L.T., for technical work, and to Mr. A. Finnie who prepared the photographs.

REFERENCES

Babes, V., and Starcovici, C. (1912): *C. R. Soc. Biol. Paris, 73, 229.*

Batten, F. E. (1913): *Proc. roy. Soc. Med., 6,* 114.

Batten, F. E., and Holmes, G. (1908): *Vet. J., 15,* 179.

Campbell, R. S. F., Martin, W. B., and Gordon, E. D. (1955): *Vet. Rec., 67,* 708.

Carougeau (1898): Quoted by Marchand and Petit (1912). *Rec. Méd. vét., 89.*

Cerletti, U. (1912): *Z. ges. Neurol. Psychiat., 9,* 520.

Cohrs, P. (1951): *Dtsch. tierärztl. Wschr., 58,* 129.

Cordy, D. R. (1942): *Cornell Vet., 32,* 11.

De Monbreun, W. A. (1937): *Amer. J. Path., 13,* 187.

Dexler, H. (1904): *Mschr. Psychiat. Neurol., 13,* 97.

Dunkin, G. W., and Laidlaw, P. P. (1926): *J. comp. Path., 39,* 201, 213 and 222.

Fankhauser, R. (1951): *Schweiz. Arch, Tierheilk., 93,* 715 and 796.

Flexner, S., and Clark, P. F. (1913): *J. exp. Med., 17,* 577.

Frauchiger, E., and Walthard, K. M. (1935): *Arch. wiss. prakt. Tierheilk., 69,* 231.

Gallego, A. (1928): *Z. Infektkr. Haustiere, 34,* 38.

Gilliespie, J. H., and Rickard, C. G. (1956): *Amer. J. vet. Res., 17,* 103.

Goldberg, S. A., and Volgenau, R. G. (1925): *Cornell Vet., 15,* 181.

Hurst, E. W. (1952): Multiple Sclerosis and the Demyelinating Diseases. Experimental Demyelination in Relation to Human and Animal Disease. Baltimore: The Williams and Wilkins Co.

Hurst, E. W., Cook, B. T., and Melvin, P. (1943): *Aust. J. exp. Biol. med. Sci., 21,* 115.

Innes, J. R. M. (1949): *Vet. Rec., 61,* 73 and 173.

Innes, J. R. M. (1949): *Brit. vet. J., 105,* 99.

Kantorowicz, R., and Lewy, F. H. (1923): *Arch. wiss. prakt. Tierheilk., 49,* 137.

King, L. S. (1939): *Arch. Path., 28,* 151.

Koprowski, H., Jervis, G. A., James, T. R., Burkhart, R. L., and Poppensiek, G. C. (1950): *Amer. J. Hyg., 51,* 63.

Kriesel, H. R. (1938): *Cornell Vet., 28,* 324.

Lauder, I. M., Martin, W. B., Gordon, E. D., Lawson, D. D., Campbell, R. S. F., and Watrach, A. M. (1954): *Vet. Rec., 66,* 607 and 623.

Lentz, O. (1907): *Zbl. Bakt., 42* (Ref. Suppl.), 126.

Lumsden, C. E. (1951): *Brit. med. J., 1,* 1035.

Marchand, L., and Petit, G. (1912): *Rec. Méd. vét., 89,* 1, 93 and 235.

Marinesco, G., Draganesco, S., and Stroesco, G. (1933): *Ann. Inst. Pasteur., 51,* 215.

Matsulevich (1884): *Arch. Vet. Nauk. 14,* 1. Quoted by Green, *et al., Amer. J. Hyg.* (1930), *12,* 109.

McGovern, V. J., Steel, J. D., Wyke, B. D., and Dodson, M. E. (1950): *Aust. J. exp. Biol. med. Sci., 28,* 433.

McGowan, J. P., and Dawson, J. W. (1914) : *J. Path. Bact., 19,* 182.
McIntyre, A. B., Trevan, D. J., and Montgomerie, R. F. (1948) : *Vet. Rec., 60,* 635.
Moulton, J. E., and Brown, C. H. (1954) : *Proc. Soc. exp. Biol. N.Y., 86,* 99.
Nicolau, S. (1935) : *C. R. Soc. Biol. Paris, 119,* 269.
Perdrau, J. R., and Pugh, L. P. (1930) : *J. Path. Bact., 33,* 79.
Peters, G., and Yamagiwa, S. (1936) : *Arch. wiss. prakt. Tierheilk., 70,* 138.
Pierret (1882) : *Lyons Med. vet.,* Quoted by Green, *et al.* (1930). *Amer. J. Hyg., 12,* 109.
Posrednik, F. I. (1930) : *Z. Infektkr. Haustiere, 38,* 135.
Potel, K. (1951) : *Exp. Veterinärmed., 4,* 44.
Potel, K. (1954) : *Schweiz. Arch. Tierheilk, 96,* 260.
Potel, K. (1955) : *Dtsch. tierärztl. Wschr., 62,* 9.
Pugh, L. P. (1948) : *Vet. Rec., 60,* 639.
Ribelin, W. E. (1953) : *Amer. J. vet. Res., 14,* 96.
Röhrer, H. (1951) : *Exp. Veterinärmed., 4,* 116.
Roman, B., and Lapp, C. M. (1925) : *J. Amer. vet. med. Ass., 66,* 612.
Rubarth, S. (1947) : *Acta path. microbiol. scand.* (Suppl. LXIX).
Sanfelice, F. (1915) : *Zbl. Bakt.* (Abt. I), *76,* 495.
Scheitlin, M., Seiferle, E., and Stunzi, H. (1951) : *Schweiz. Arch. Tierheilk., 93,* 91.
Scherer. H. J. (1944) : *Verglieichende Pathologie des Nervensystems der Säugetiere.* Leipzig.
Seifried, O. (1931) : *J. exp. Med., 53,* 277.
Singleton, W. B., and Hunter, F. E. (1956) : *Vet. Rec., 68,* 21.
Sinigaglia, G. (1913) : *Pathologica., 103,* 107.
Sjolte, I. P. (1947) : *Skand. Vettidskr., 37,* 350.
Standfuss, R. (1908) : *Arch. wiss. prakt. Tierheilk., 34,* 109.
Steel, J. D., and Whittem, J. H. (1950) : *Aust. vet. J., 26,* 197.
Taty and Jaquin (1898) : Quoted by Marchand and Petit (1912). *Rec. Méd. vét., 89.*
Trevan, D. J. (1953) : *Proc. roy. Soc. Med., 46,* 890.
Triboulet (1893) : Quoted by Marchand and Petit (1912). *Rec. Méd. vét., 89.*
Watson, E. A., and Plummer, P. J. G. (1942) : *Amer. J. vet. Res., 3,* 350.
Whittem, J. H., and Blood, D. C. (1950) : *Aust. vet. J., 26,* 73.
Winqvist, G. (1950) : *Nord. Vetmed., 2,* 367.
Wood (1885) : Quoted by Green, *et al.* (1930). *Amer. J. Hyg., 12,* 109.

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ILLUSTRATIONS

Fig. 1—Mid-brain: Early acute encephalitis. There is a reaction by subpial glia and slight activation of meningeal cells. Hæmalum-phloxin-tartrazine. x 110.

Fig. 2—Cerebellum: Acute gliosis in an area of white matter adjoining the pia mater. Hæmalum-phloxin-tartrazine. x 35.

Fig. 2a—Cerebellum: Acute gliosis in superficial white matter with rarefaction of myelin. Vacuolation is well marked. Loyez' hæmatoxylin. x 35.

Fig. 3—Cerebellum: Early gliosis. All forms are swollen. Distorted microglial nuclei can be seen and oligodendroglial cytoplasm appears as a clear halo. Hæmalum-phloxin-tartrazine. x 420.

Fig. 4—Cerebellum: Vacuolated compound granular corpuscles in demyelinated lesion. Their cytoplasm gives a positive PAS reaction. Periodic acid-Schniff. x 420. Green filter.

Fig. 5—Cerebellum: Normal astrocytes. Cajal's gold-sublimate. x 420.

Fig. 5a—Cerebellum: Acute gliosis. The astrocytic bodies are swollen and their fibres disintegrating. Cajal. x 420.

Fig. 6—Cerebellum: Astrocytic "scarring" shown by a concentration of pale ovoid nuclei with inconspicuous cytoplasm. A relatively inactive lesion in a case of eight weeks' duration. Hæmalum-phloxin-tartrazine. x 420.

Fig. 7—Cerebellum: Acidophilic, cytoplasmic inclusions of various sizes apparently lying in astrocytes. Some are surrounded by a halo. Hæmalum-phloxin-tartrazine. x 1100. Green filter.

Fig. 8—Cerebellum: Pale acidophilic inclusions in the nuclei of astrocytes. In several nuclei, darker nucleoli can also be distinguished. Hæmalum-eosin. x 1100.

Fig. 9—Cerebellum: Diffuse gliosis and vacuolation in the white matter of the folia. Hæmalum-eosin. x 35.

Fig. 10—Cerebellum: Demyelination and vacuoles in folia. Loyez' hæmatoxylin. x 35.

Fig. 11—Vacuolation in a myelinated area. Some, but not all, of the spaces are clearly related to oligodendrocytes. Loyez' hæmatoxylin. x 420.

Fig. 12—Internal capsule. There is general acute swelling of oligodendrocytes and numerous free masses of PAS positive material. Periodic acid-Schiff. x 110.

Fig. 13—Ependyma. Many acidophilic cytoplasmic inclusions are present, some with haloes. Hæmalum-phloxin-tartrazine. x 1100.

Fig. 14—Temperal cortex. "Acute capillaritis" vessels of different grades show swelling of their walls and a slight increase of cells around them. Hæmalum-eosin. x 110.

Fig. 15—Temperal cortex. Acute capillaritis, showing swelling and mitotic division among mural cells. Hæmalum-eosin. x 420.

Fig. 16—Thoracic cord. There is dense perivascular "cuffing" by lymphocytes, some of which spill into the adjacent tissue. There is also an increase of glia nearby. Giemsa. x 420.

Fig. 17—Thalamus: A general glial reaction and demyelination are accompanied by perivascular infiltration by lymphocytes and other mononuclear cells. Hæmalum-eosin. x 110.

Fig. 18—Thalamus: Perivascular infiltration of a congested meningeal vein. The underlying tissue also shows a cellular reaction. Hæmalum-eosin. x 420.

Fig. 19—Cerebral cortex. Five terminal colonies (pseudocysts) of toxoplasma situated among chromatolytic neurones. Wright and Craighead. x 420.

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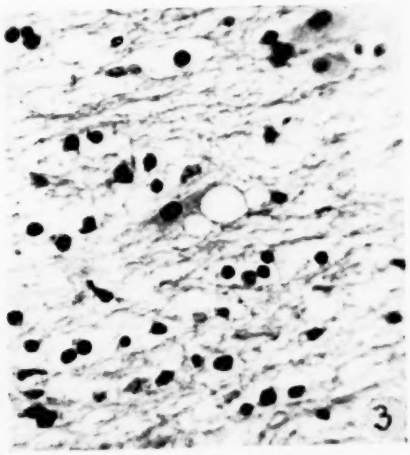
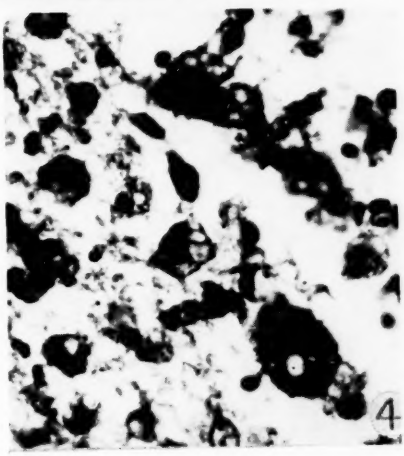
PLATE I





(*Article by Campbell, page* 143)

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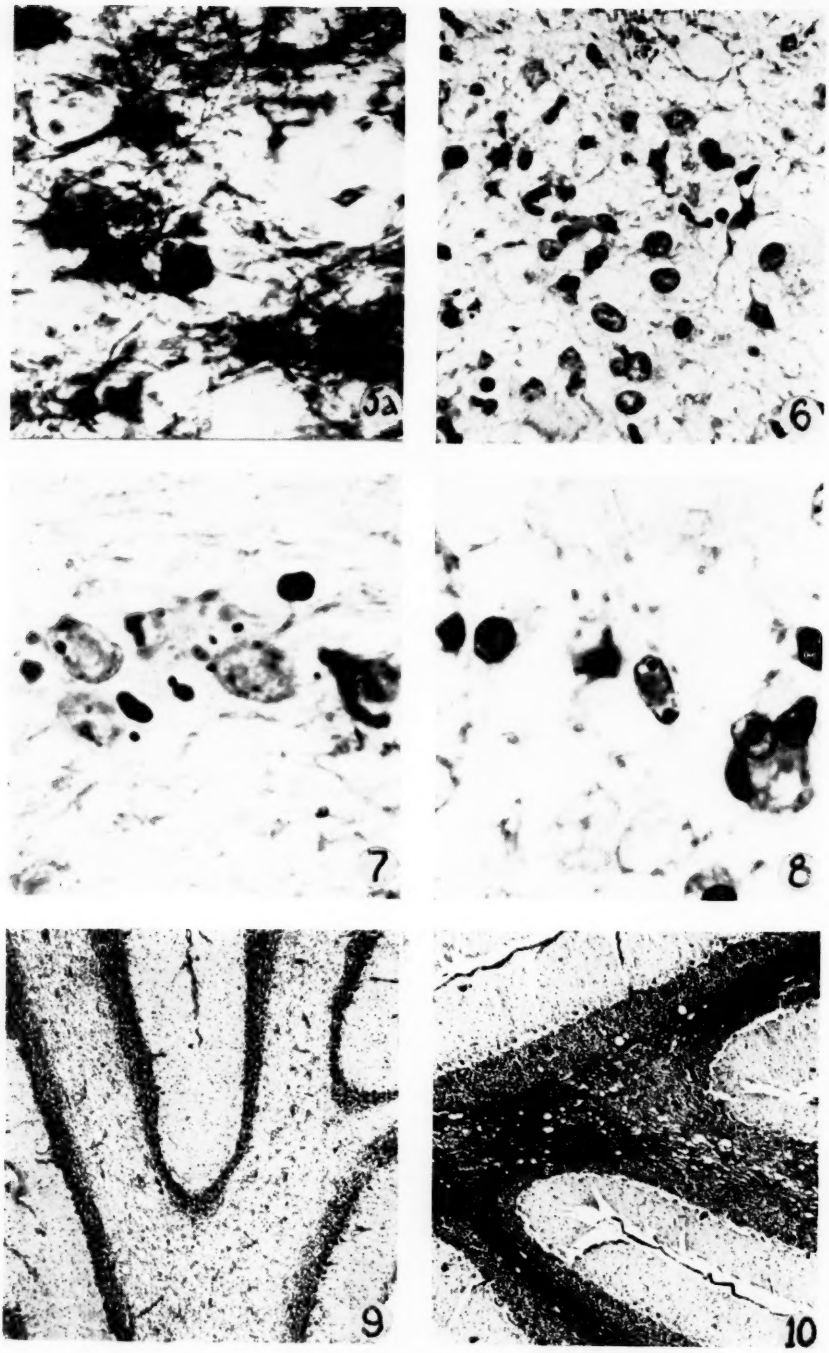

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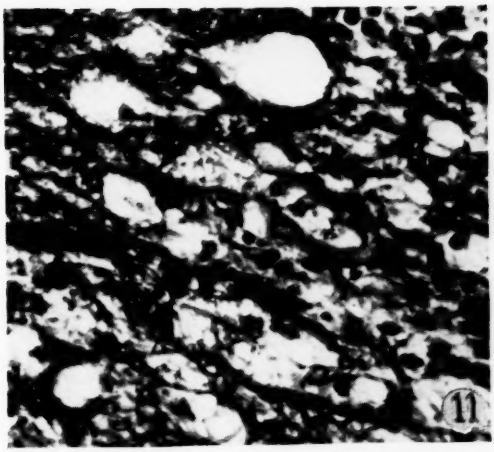

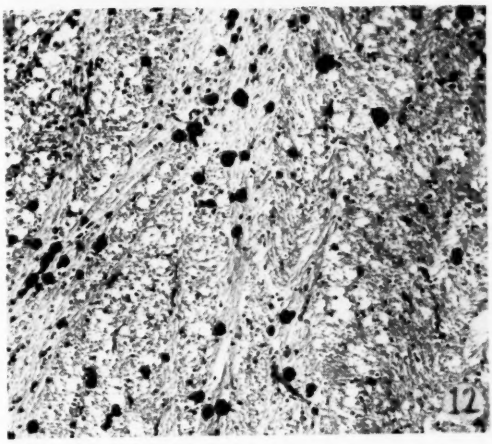

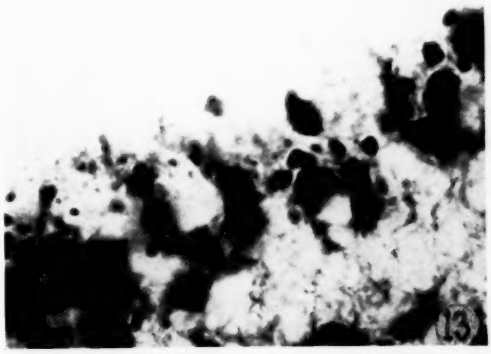

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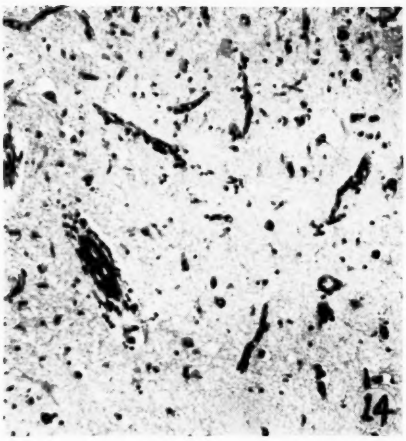

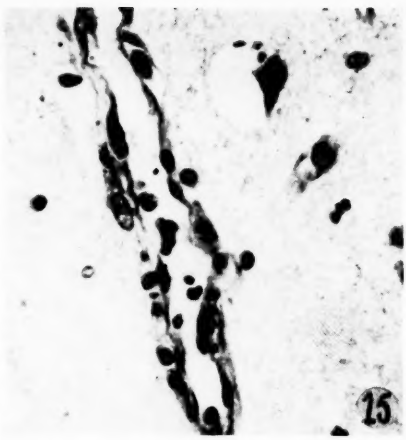

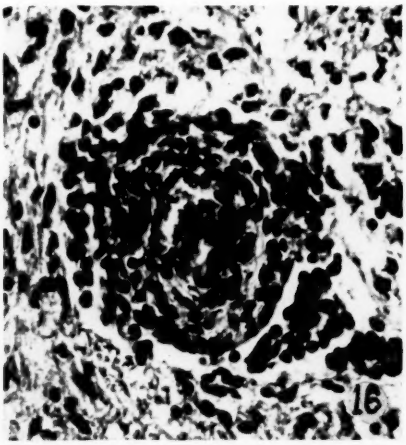

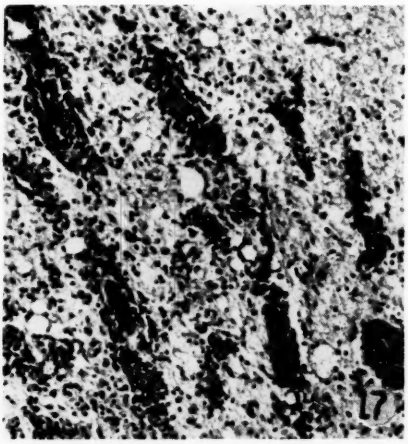

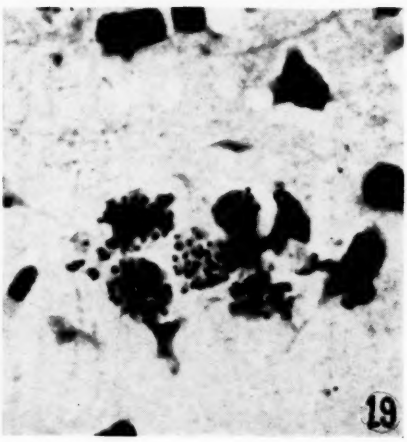

(*Article by Campbell, page* 143)

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SEDATIVES AND ANÆSTHETICS FOR BABOONS

By J. NEWSOME and D. L. H. ROBINSON
from Medical Research Council Group for Research in Bilharzia

WHEN it is necessary to handle a strong and potentially dangerous laboratory animal there is usually much discussion on the ways and means. It is the common experience that sedatives and anæsthetics vary greatly in their effectiveness on individual animals and the best method for any one species is evolved only slowly.

During the last eight years many methods of handling baboons (*Papio hamadryas* and *P. papio*) have been tried, and eventually a fairly satisfactory *modus operandi* has been adopted. The animals were of all ages; they weighed up to 26 kg., and were used to maintain *Schistosoma mansoni* infection. They were subjected to infection by water containing cercariæ, to rectal biopsy, liver biopsy, heart puncture with removal of up to 350 c.c. blood and venipuncture; sedatives and anæsthetics were also used to examine wounds occasioned when fighting and to separate mothers from babies. Some fifteen different substances have been used by different techniques. The results are summarised briefly in this note.

Morphine Hydrochloride.

This was given by mouth and by injection. The only effect of doses up to 1 grain was very severe skin irritation, especially of the palms and soles, and loss of appetite. There was no noticeable sedation.

Amidone Hydrochloride (*Physeptone*).

The manufacturers* suggested a dose of 80 mg. intramuscularly. This was given to several animals of different sizes. Skin irritation was intense but it was relieved somewhat by anti-histamine drugs. Some animals became docile for short periods but one, which had completely recovered for 2½ hours from an earlier injection of pentobarbitone sodium, became unconscious and died. No further trials were made of amidone.

Chloral Hydrate.

One gram or more was given by mouth in many different ways. The animals always discovered the drug and discarded or refused its vehicle. All tablets were bitten open and the bitter taste prevented further attempts to use chloral.

Chloralose.

As this compound of chloral and glucose is almost tasteless it was given in a hollowed-out banana and as a rule it was taken well. Doses of 1 to 1.5 g. were without effect, but when 3 g. was given to an animal of 15 kg. and 4 g. to one of 25 kg. heavy sedation was produced in 30 minutes and lasted up to three

* Burroughs Wellcome & Co., Ltd.

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hours. The animals, although semi-conscious and easily handled, suffered from rhythmical clonic spasms of the limbs and head unless they were held very firmly, and this interfered with experimental procedures. Salivation and excessive sweating were observed also. Chloralose in pill form was then tried but the large dose, the spasms and the sweating prevented the drug from being of much use.

Carbromal (*Adalin*).

Some slight effect was seen on one occasion after a dose of 20 grains in four tablets, but there was no effect on other occasions.

Sulphonal.

Doses of up to 12 grains were secreted in figs and given to five animals with no effect.

Butobarbitone (*Soneryl*).

The bitter taste made Soneryl difficult to administer, but up to $4\frac{1}{2}$ grains were taken without effect.

Pentobarbitone Sodium (*Nembutal*).

This could not be given by mouth but was used frequently by intramuscular injection until very recently. It was always difficult to decide on the dose. Veterinary Nembutal (1 grain/c.c.) was used, and the doses given were recorded on each animal's notes. On some occasions a baboon given the same dose as before passed into a deep coma and stopped breathing. Several animals were lost. Sometimes it was necessary to increase the dose on each occasion, but almost as often the dose had to be reduced. There was never any doubt, after several hundred injections of nembutal, that it was an unsuitable and unpredictable drug for valuable animals when given alone. Even when combined with intramuscular paraldehyde (q.v.), unexpected emergencies kept arising. It was used once or twice as a premedication for ether anæsthesia but was found to be unreliable. We have not used nembutal and ether after nitrous oxide induction which in other hands has given quite good results.

Hexobarbitone (*Evipan*).

Sugar-coated tablets were accepted readily, but four such tablets containing 16 grains of Evipan were without effect. Usually five or six tablets of any kind given at one time were not eaten by the baboons whose interest in them waned after the fourth.

Amylobarbitone Sodium (*Sodium Amytal*).

As is observed with other barbiturates, sodium amytal had no effect in doses of 6 grains when given by the mouth.

Somnifaine.

No satisfactory dosage could be devised. Similar animals receiving 2, 3 and 4 c.c. showed, respectively: no effect, unconsciousness for 23 hours, and coma and death in four hours.

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Paraldehyde.

It had a definite calming effect when given intramuscularly in doses of up to 5 c.c. It was most useful when given with nembutal. An animal requiring 3.5 c.c. (3½ grains) of Nembutal could often be anæsthetised by 2 c.c. of Nembutal and 3 c.c. paraldehyde given intramuscularly at the same time. For a long time this was the method of choice for minor procedures. However, in spite of keeping careful record of the doses used, unexpected emergencies and fatalities occurred sometimes.

Chlorpromazine (Largactil).

This seemed likely to be most valuable as a sedative, but the baboons varied greatly in their response. Doses of up to 3.5 mg./kg. were given, and the animals became apathetic and slow. They could not be handled with any ease. One large male received 2.5 mg./kg. daily on four consecutive days and his reaction was much less on the third and fourth days. The action of the drug (given intramuscularly) was too slow, its maximum effect occurring at 2½ to six hours after injection instead of at the expected time of 40 minutes. This unreliability of largactil in primates has been confirmed elsewhere.

Ether and Chloroform.

Open ether was used extensively, but induction was never easy. If nembutal was used beforehand, the breathing was nearly always unsatisfactory, and without preliminary sedation there was much struggling. Ethyl chloride was sometimes helpful. Baboons often needed very large amounts of ether before they relaxed and usually the difficulty was to appreciate what a great deal of ether was required. Chloroform alone was not used because the bilharzia infection was known to cause chronic liver infection. It was given with ether (1 : 3) but large amounts were still required, and it was felt that ether alone was equally effective and safer, especially if the anæsthesia was required to last for half an hour or more.

Nitrous Oxide.

The use of nitrous oxide has now supplanted the other methods. The baboon is given gas in a small anæsthetic box and as soon as it drops (usually in three minutes or less) some ether is blown into the box. Then the animal is removed and given ether, fed by compressed air to a mask.

The present method.

The caged baboon is introduced into a wooden cage (about 2 ft. cube), into which it goes readily. The roof of the cage descends gently upon the animal and closes it into the anæsthetic chamber, connected to the cage. This chamber measures 2 x 2 x 1½ ft., and there is a Perspex window in its sliding door.

Gas is passed from the cylinder through a veterinary ether apparatus* with the ether and rebreathing bag taps turned off. It passes along the corrugated tube and into the chamber. When the animal is down, ether is given sometimes for one to two minutes before removal. The gas is then turned off, and air from

* Oxygen-Ether Apparatus, British Oxygen Co. Ltd.

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a pump connected to the ether apparatus. The corrugated tube is disconnected from the anæsthetic box and joined to the mask. Then the baboon is removed and given ether and air through the mask. The human stages of anæsthesia are rarely seen and the corneal reflex and the breathing rhythm are uncertain guides to the depth of anæsthesia. However, the animal can be " kept under " for minor procedures with ease, and with practise abdominal relaxation can be maintained for long periods.

Comment.

These animals have proved difficult to dose by mouth, and intramuscular injection often provokes something of a battle. The alternative of inhalation anæsthesia was explored some years ago and a large box was built, into which the animals could be introduced easily. Ether was given on pads of cotton (which was quite unsatisfactory) or by blowing compressed air, and later nitrous oxide, through the ether bottle. The results were encouraging but not reliable. It was not until the smaller type of box, designed and used by Mr. Oliver G. Jones of the Zoological Society of London, was made that reliable anæsthetics seemed possible. Even with this method the mistake was made of continuing the anæsthetic by blowing gas and air through the ether to the mask. Animals frequently stopped breathing and one died. Baboons seem to be very sensitive to gas, and there was no further trouble when the ether was given with compressed air alone.

The vagaries of individual baboons in their reaction to the various compounds which had been tried were very pronounced, and until now it has been unwise to recommend any particular method of anæsthesia. With some relief, therefore, it is now possible to say that rapid nitrous oxide induction followed by ether and air has proved easy, safe and not frightening for baboons and for a chimpanzee. It is a great convenience to be able, without frightening or even handling the animal, to have it on a table under light ether anæsthesia in about five minutes from the time it reaches the laboratory.

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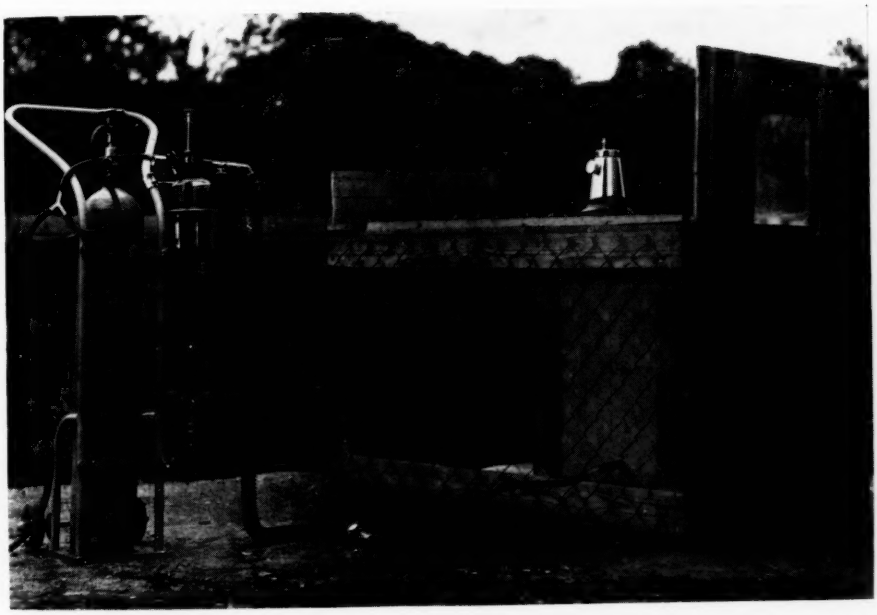

The anæsthetic apparatus.

(*Article by Newsome and Robinson, page* 163)

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A cow in the state of submission.

(*Article by Fraser, page* 167)

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THE STATE OF SUBMISSION IN CATTLE

By A. F. FRASER, M.R.C.V.S.
Lanark

VETERINARY SURGEONS and stock owners are familiar with the problem of the cow which is down in its box or stall and refuses to arise. The condition is one in which there is no immediate pathological cause such as paralysis or some metabolic upset such as hypocalcæmia. It may follow an illness such as milk fever, and a cow which has made a full recovery and is again in normal physical health may refuse to get up. The problem presented for solution is of a psychological rather than of a pathological nature.

Sometimes a satisfactory response may follow the application of external stimuli of a pseudo punitive character. The reflex escape response which is induced may cause the beast to arise. If this does occur then as a rule no further trouble is experienced. There are cases in which the escape response cannot be induced and the beast passes into a state of submission and assumes the submissive posture.

This posture has been mentioned by me (Fraser, 1952) as appearing in patients affected by post-parturient paralysis. Lorenz (1953) has described submissive gestures in dogs and turkeys, and he noted that the posture may be assumed by other species.

The object of my remarks is to discuss the submissive state as seen in cattle and to describe the characteristic posture which is assumed. In a typical case the head is thrust forward vigorously in full extension and the ventral aspects of the mandibular and cervical regions are laid close to the ground. The extension of the head and the pushing out of the muzzle is effected suddenly and it is sustained for as long as the punitive stimuli continue to disturb. The vigour of extension appears to vary directly according to the discomfort provoked by the stimuli. When full extension has occurred and the severity of the stimulus be increased, the picture may be exaggerated by side to side swaying movements of the head. Exophthalmus may be noted also. If the stimulus be stopped the reaction is revoked quickly. If the stimulus be repeated on a number of consecutive occasions the submissive state becomes more strongly asserted until a point can be reached when an implied threat is sufficient to evoke the typical response in behaviour.

Although the significance of the posture may be not recognised, most observers who have practical experience of this particular trouble know that when the point of the above display has been reached one cannot anticipate any co-operation from the subject. The state of submission is a complete barrier to any other response to the particular stimulus applied. It is a state which may lose its specificity to a particular stimulus if it is strengthened by frequent assertion. This loss of specificity by the submissive state leads to the induction of instant submission by almost any disturbance. This expanded elicitation of submission lessens the chances of recovery. When the submissive posture is displayed the particular stimulus should be withdrawn and not repeated. Alternative external

stimuli may be tried which do not make use of the neural pathways on which a psychological barrier has been formed.

In attempting to induce an unwilling cow to get up, one of two results may be expected: (a) we may elicit an escape response in the cow and by her co-operation achieve success; (b) we may provoke a state of submission, get no co-operation from the patient, and all our efforts be in vain.

REFERENCES

Fraser, A. F. (1952): *Vet. Rec., 64,* 27, 400.
Lorenz, K. L. (1953): King Solomon's Ring. Reprint Society Ltd.

HÆMOLYTIC *BACT. COLI* IN THE "BOWEL ŒDEMA" SYNDROME

PART. I—An attempted investigation into the pathogenicity of the organism for experimental animals.

By M. GITTER, M.R.C.V.S.

ATTEMPTS have been made to assess the pathogenic significance of hæmolytic strains of *Bact. coli* which have been isolated in pure or almost pure culture from the intestinal tract of pigs dying of "bowel œdema."

In 1951 Hudson, Huck and Shand tried to elucidate the relationship between "bowel œdema" syndrome and hæmolytic *Bact. coli.* The supernatant fluid obtained by high-speed centrifugation of hæmolytic *Bact. coli* cultures was injected intravenously into one pig and nine mice. No effect was produced in the pig, but eight of the mice showed marked nervous symptoms (circling movements and paraplegia) and diarrhœa. The controls injected intravenously with sterile peptone broth showed no change in their behaviour. All mice were killed and hæmolytic *Bact. coli* were recovered from all the spleens, but no growth was obtained from the brains. The supernatant fluid was not bacteria free. Histological examination of brains showed no change indicative of an infective agent, and intracerebral transmissions of brain tissue into mice produced no ill effect.

In August, 1954, the author recovered hæmolytic *Bact. coli* (strain A) in pure culture from the intestine and the œdematous lung of a pig from a field outbreak of "bowel œdema."

Cultural Characteristics.

Circular, low, convex, smooth greyish-white colonies about 1-2 mm. in diameter with an entire edge and a wide zone of ß hæmolysis were observed on 5 per cent ovine blood agar after 24 hours incubation at 37° C. In peptone broth there was an abundant growth with a uniform turbidity, increasing after 24 to 72 hours' incubation; a slight, powdery deposit occurred which dispersed on shaking.

Morphology. Gram negative cocco-bacilli with parallel sides and rounded ends.

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Biochemical reactions. The organism produced acid and gas from adonite, arabinose, dextrin, galactose, glycerol, lactose, lævulose, maltose, mannite, mannose, rhamnose, salicin, sorbite, trehalose and xylose. It acidified litmus milk, formed indole and reduced nitrates to nitrites. The methyl red reaction was positive and the Voges Proskauer negative. No change was produced in dulcite, inosite, inulin, raffinose and sucrose, and the organism did not grow with citrate as the only source of carbon.

Animal Inoculations.

Six mice and one pig were injected with the culture isolated from the lungs. The mice received 0.2 c.c. of 6½-hour peptone broth culture; three intravenously, one intraperitoneally, one subcutaneously and one intranasally.

The three mice which received the culture intravenously showed marked nervous symptoms, in one case within one hour and in the other two within 18 to 24 hours (jumping, holding head to one side, somersaults, pedalling and convulsions). All died within 18 to 24 hours and post-mortem examination revealed congestion of the internal organs, with marked enlargement of the spleen. Two cases showed hæmoglobinuria. On bacteriological examination hæmolytic *Bact. coli* were isolated from all internal organs. The remaining mice remained healthy.

Two mice were used as controls and they received intravenously 0.2 c.c. of a six-hour peptone broth culture of a non-hæmolytic strain of *Bact. coli* isolated from a rectal swab of a normal pig. These mice remained normal.

Pig No. 9874, about 10 to 12 weeks old, in fairly good bodily condition, was injected intravenously on August 20, 1954, with a mixture of 5 c.c. of a six-hour and 5 c.c. of a 24-hour peptone broth culture of strain "A." On the following day the pig was recumbent and there was marked dyspnœa; the temperature was 104.2° F. Two days later the temperature rose to 105.6° F. and the pig remained recumbent till the following day, when the temperature began to fall. Four days after injection the animal attempted to get up when aided. During the first three days there was complete anorexia, but the appetite returned during the following four to five days, during which a gradual recovery took place, and the animal was normal by the 10th day after injection.

A similar experiment was made with another strain of hæmolytic *Bact. coli* (strain B) isolated from the colon of a pig from another field outbreak of "bowel œdema." This strain was indistinguishable from strain A by cultural and morphological characteristics. It differed in its biochemical reactions by failing to produce any change in adonite and salicin and by producing acid and gas from sucrose. Out of three mice injected intravenously with a six-hour peptone broth culture, two died within 24 to 36 hours. No nervous symptoms were noted. The remaining mice and controls remained normal.

Pig No. 9764 was given intravenously (on August 24, 1954) a mixture of 5 c.c. of a six-hour and 5 c.c. of a 24-hour peptone broth culture of strain B. Next day the temperature rose to 105.6° F., the animal was dull, and refused all food. Pyrexia persisted for seven days with the temperature ranging from 104.2° F. to 106.4° F. Complete anorexia was observed on the first two days

after injection with the appetite gradually returning in spite of an elevated temperature, and becoming normal by the eighth day after injection. No other symptoms were seen.

Both pigs (Nos. 9874 and 9764) were killed and no lesions were observed post-mortem.

Additional intravenous injections of strain B to mice gave varying results, and in general a small proportion of mice died. Subcutaneous and intraperitoneal injections were always without effect.

Hæmolytic *Bact. coli* were recovered from all organs of three day old piglets in an outbreak of catarrhal enteritis. The culture, strain "C," was found to be morphologically, culturally and biochemically indistinguishable from strain "A." Three mice were injected intravenously with 0.2 c.c. of a six-hour broth culture and all died. On post-mortem examination there was generalised congestion of the internal organs and marked enlargement of the spleen; hæmoglobinuria occurred in two. Intraperitoneal and subcutaneous injections, even in large doses, were harmless. Control mice injected intravenously with 0.2 c.c. of a six-hour broth culture of a non-hæmolytic *Bact. coli* isolated from the rectal swab of an apparently healthy pig remained unaffected.

Discussion

According to Parnas *et al.* (1950), the maximum production of hæmolysis by porcine strains of *Bact. coli* takes place during the active growth of the cultures. An association between hæmolysis and virulence of *Bact. coli* for experimental animals is suggested by authors whom they quote. Since maximum virulence of the organisms may coincide with the maximum production of hæmolysis, it was arranged that young cultures should be used in the above experiments. The results obtained with the three strains tend to support the findings of Parnas *et al.* (1950) that strains of *Bact. coli* recovered from the parenchymatous organs are more virulent than those isolated from the intestinal tract. Strains A and C appeared to be more virulent to mice than strain B, which was recovered from the colon; the intravenous injection of strain A produced more pronounced symptoms in a pig than strain B.

Conclusions

The regular recovery of hæmolytic *Bact. coli,* usually in pure culture from the intestines and sometimes also from other organs of pigs dying of "bowel œdema," suggests that further studies should be made of the part played by this organism in the "bowel œdema" syndrome. The identification of strains by serological means and the possible production of toxins are some of the problems worth investigating.

It should be noted, however, that strains of hæmolytic *Bact. coli* have also been recovered from pigs in conditions other than the "bowel œdema" syndrome.

Acknowledgment

The help received in the course of this investigation from Mr. R. A. Huck, B.Sc., M.R.C.V.S., is gratefully acknowledged.

REFERENCES

Hudson, J. R., Huck, R. A., and Shand, A. (1951): Unpublished work.
Parnas, J., *et al.*, (1950): *Ann. Univ. M. Curie-Sklodowska, 5,* 20.

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THE MUCUS ANTIGENS OF *ESCHERICHIA COLI*

By T. A. REES

Department of Pathology, Royal Veterinary College, London

DESCRIPTIONS of mucoid and non-mucoid colonies of coliform organisms are to be found in some of the earliest records of the serological work on *Escherichia coli*. More recently, the mucus secreted by these organisms has been examined to determine whether or not it has a serological identity of its own.

Henriksen (1949, 1950, 1953), working in Scandinavia, obtained results which suggested the existence of a mucus or "M" antigen which is common and apparently uniform throughout the Escherichia group. He prepared extracts of mucus from suitable strains by means of centrifugation or filtration of saline suspensions. Using the precipitin technique, he found cross-reactions of a low order between all the mucus extracts and antisera prepared against the homologous strains. In his second and third papers he describes the existence of serological relationships between the mucus secretions of *E. coli* and of *S. paratyphi B* and between *E. coli* and certain Klebsiella types. He concluded that this M antigen is a substance of low and uncertain antigenic effect and that it is probably uniform in all strains of *E. coli*.

In contrast with this, Wilkinson, Duguid and Edmunds (1954) suggested that the mucus polysaccharides of the strains of *E. coli* and *K. aerogenes* examined by them are serologically identical with their capsular polysaccharides. These are the "K" antigens of the respective strains and this implies that there are as many antigenic types of mucus as there are "K" antigens.

Taylor and Charter (1955) found a mucoid strain of 0.128 B12 which apparently possessed a morphological capsule in addition to, but different from, its surface or K antigen. They report that "although no detailed investigation of the slime and capsule was made, the tests which were done suggested that the two were either the same or very closely related in their serological reactions."

There seemed to be a great deal of confusion with regard to the true antigenic nature of the mucus secreted by *E. coli*, and the work described below was undertaken in an attempt to clarify the situation.

Methods

Strains Studied. Nine strains of *E. coli* were selected on the basis of their antigenic structure; the accompanying list gives the relevant data. It will be seen that three strains were non-motile, two (RVC 1798 and RVC 118) had a common somatic antigen but differed in their surface antigen, whilst two (RVC 118 and RVC 1616) shared a surface antigen but differed in their somatic ones.

Strain	*Antigens* O (somatic)	K (surface)	
RVC 1798	9	A	Somatic antigen identical with that of RVC 118. Non-motile.
RVC 118	9	A'	Somatic antigen identical with that of RVC 1798. Surface antigen identical with that of RVC 1616. Non-motile.
RVC 1616	101	A'	Surface antigen identical with that of RVC 118.
RVC 101	35	K?	Non-motile.
RVC 330	78	B?	
RVC 1034	?	?	Contains no known antigen.
RVC 1787	137	79(L)	
—	86	B7	
—	126	B16	

Preparation of Mucus. Each strain was grown on 0.1 per cent glucose agar at 22° C. for several days in order to increase the production of mucus; four days was long enough with most. The growth was removed from the surface of the medium with a glass rod, suspended in sterile saline and gently agitated to produce a uniform suspension. The suspension was left for 30 minutes at 37° C. to allow the mucus to disperse. This was followed by centrifugation to remove the bacterial bodies, the supernatant fluid being pipetted off and kept without preservative as a solution of mucus. When not required for any length of time, mucus samples were kept frozen at —20° C. without detriment to their antigenicity.

Antisera. These were prepared by the intravenous inoculation into rabbits of saline suspensions of living bacteria grown on glucose agar. No preservative was added to the antisera and, when not required, they were kept in the deep-freeze as in the case of mucus solutions.

Precipitin tests. Each sample of mucus was tested in serial dilutions against constant amounts of all nine antisera. The tests were made in Dreyer's tubes, each tube containing 0.2 ml. of the antigen diluted in saline and 0.2 ml. of undiluted serum. The range of final dilutions of the mucus extended from 1 :2 to 1 :2048. After being left overnight at room temperature, the tubes were centrifuged at 2,000 r.p.m. for 30 minutes and examined with a hand lens; according to the technique employed by Henriksen. Serum and antigen controls were also made and examined in a similar manner.

Results (see table)

It will be seen that there are two orders of reaction taking place. Homologous reactions, and those between RVC 118 and RVC 1616, are characterised by a precipitate in high antigen dilutions. These precipitates appeared almost immediately in the tubes containing the higher antigen concentrations.

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Heterologous reactions are diffuse and were demonstrated only after the antigen-antibody mixture had been centrifuged after standing all night. Precipitation rarely occurred in antigen dilutions greater than 1 : 128.

Results of precipitin tests between preparations of mucus from selected strains and their homologous sera.

MUCINS	SERA								
	RVC 101	RVC 118	RVC 330	RVC 1034	RVC 1616	RVC 1787	RVC 1798	0.86, B7	0.126, B16
RVC 101	1024	32	32	128	64	32	2	32	128
RVC 118	32	1024	64	256	1024	32	8	32	16
RVC 330	32	64	1024	256	128	32	4	64	32
RVC 1034	64	64	64	2048	128	128	32	64	128
RVC 1616	32	1024	32	64	1024	16	8	32	4
RVC 1787	128	128	256	128	16	1024	4	128	16
RVC 1798	16	64	16	128	256	8	1024	2	64
0.86, B7	128	8	16	128	128	64	8	1024	16
0.126, B16	16	32	128	128	64	32	4	128	512

Figures are the reciprocals of the highest antigen-dilutions in which precipitation occurred.

Discussion

The occurrence of precipitates in high antigen dilutions in tests with homologous antisera indicates the specific nature of these reactions; this specificity occurs also in the cross-sections between the mucus and antisera of strains RVC 118 and RVC 1616.

Were the high order of the homologous reaction due to the interaction between somatic antigen present in the mucus and the serum somatic antibodies, it would be logical to expect a similar result in the cross reactions of RVC 118 and RVC 1798 since they have the same somatic antigens but different K antigens. This, however, is not the case. If it were due to flagellar antigen, then it would be difficult to account for the high homologous reactions in the cases of strains RVC 101, RVC 118 and RVC 1798, all of which are non-motile. The mucus antigen described by Henriksen would not be expected to give reactions of such a high order; nor, in fact, any specific reactions at all. The remaining antigen is the K or surface antigen and that it is responsible for these high specific reactions gains confirmation from the cross-reactions between RVC 118 and RVC 1616 which have identical K but different somatic antigens.

The cross-reactions which occurred in low dilutions in all cases have to be accounted for on a different basis. That the somatic antigens are not responsible is proven by the fact that, with the exception of RVC 118 and RVC 1798, all the strains were chosen deliberately on account of their differing somatic

antigens. Similar conclusions may be drawn from a consideration of the results with the surface antigens which, with the exception of strains RVC 118 and RVC 1616, were different. Flagellar antigens were not identified in the case of motile strains but there can be no doubt that they were not responsible for cross-reactions between the three non-motile strains. These results with low antigen dilutions appear to accord with the definition of the mucus antigen given by Henriksen.

When consideration is given to the precipitation reactions occurring in both high and low antigen dilutions, then it appears that the mucus secreted by *E. coli* has a much more complicated antigen content than was realised at first. The results discussed above support the findings of both Wilkinson, *et al.* and Henriksen in so far as they go, but it is possible that there are traces of somatic or flagellar antigens present as well. A suggestion that somatic antigen may be present was given by the results of a test made on the mucus of RVC 330 with an antiserum prepared from boiled cultures of this organism. This contained somatic antibodies only and when added to the antigen dilutions, there was slight precipitation up to a dilution of 1 : 8. No attempt was made to demonstrate the presence of flagellar antigen.

Strains of *E. coli* isolated from pathological conditions tend to be mucoid (Smith and Orcutt, 1925; Vahlne, 1945) and in calves suffering from white scours, there is also a tremendous increase in the number of these organisms in the gut (Smith and Orcutt; Carpenter and Woods, 1924). Taken together, these findings seem to suggest the release into the lumen of the intestine of a sick calf, of quantities of mucus well in excess of the normal. This mucus would, as has been shown, contain a variable amount of the K antigen. *In situ* on the bacterium, the K antigen has the function of protecting the coliform organism against the defensive mechanisms of the host (Smith, 1928; Sjöstedt, 1946). The effect, if any, of the presence of this antigen in the free state in the lumen of the intestine remains to be determined.

Summary

At least two antigenic substances are shown to be present in the mucus secreted by selected strains of *E. coli*. One, which is non-specific and probably present in all strains, appears to correspond to the "M" or mucus antigen described by Henriksen. The other is specific to individual strains and is probably identical with the surface antigens. The presence in the mucus of small somatic and flagellar elements is suggested.

Acknowledgments

I thank Professor R. Lovell for his help and encouragement in the preparation of this paper. Also Dr. Joan Taylor who supplied me with the cultures of 0.126,B16 and 0.86,B7.

This work was carried out while in receipt of a grant from the Agricultural Research Council.

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REFERENCES

Carpenter, C. M., and Wood, G. (1924): *Cornell Vet., 14,* 218.
Henriksen, S. D. (1949): *Acta path. microbiol. scand., 26,* 903.
Henriksen, S. D. (1950): *Ibid., 27,* 107.
Henriksen, S. D. (1953): *Riassunti Commun. VI. Congr. int. Microbiol., 2,* 311.
Sjöstedt, S. (1946): *Acta path. microbiol. scand. Suppl.,* 63.
Smith, T. (1928): *J. exp. Med., 48,* 351.
Smith, T., and Orcutt, M. L. (1925): *Ibid., 41,* 89.
Taylor, J., and Charter, R. E. (1955): *J. clin. Path., 8,* 276.
Vahlne, G. (1945): *Acta path. microbiol. scand. Suppl.,* 62.
Wilkinson, J. F., Duguid, J. P., and Edmunds, P. N. (1954): *J. gen. Microbiol., 11,* 59.

THE SENSITIVITY OF VARIOUS BACTERIA TO CHEMOTHERAPEUTIC AGENTS

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Introduction

THE relative potencies of sulphonamides against various animal pathogens have been studied intensively, both *in vitro* and *in vivo*. There is less information on the relative potencies of the antibiotics, so it was decided to investigate this subject, and sulphadimidine sodium was included to provide a link with other studies.

Milk was selected as the basal medium as it contains fewer inhibitory substances than infusion media against sulphonamides and penicillin, and gives a very uniform antibacterial end-point against streptococci (Brownlie, 1945; Edwards, 1949; Watts and McLeod, 1946). Again, 10 per cent of horse blood was added to the medium as it has been shown that horse blood antagonises substances which inhibit the action of sulphonamides (Maclay and Slavin, 1947).

Materials and Methods

Antibiotics Tested. Penicillin, chlorotetracycline, oxytetracycline, chloramphenicol and streptomycin were the five antibiotics selected for *in vitro* and *in vivo* tests. Calcium penicillin with a potency of 1 unit per 0.6 μg. and procaine penicillin 1 unit per 0.8 μg. were used for *in vitro* and *in vivo* tests respectively. Chlorotetracycline was available as aureomycin hydrochloride, 250 mg. per capsule; oxytetracycline as terramycin hydrochloride, 50 mg. per capsule; chloramphenicol as chloromycetin, 250 m.g. per capsule; sulphadimidine sodium as sulphamezathine $33\frac{1}{3}$ per cent solution.

In vitro. One batch of Bonlac separated milk powder was used and a 20 per cent solution was made. The solution was sterilised by steaming on three successive days, and for each experiment a given volume of milk was added to an equal quantity of melted 5 per cent agar, thus avoiding reheating of the medium. Finally, the *pH* was adjusted to 7.2. All solutions of the drugs were

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prepared fresh in distilled water for each experiment. Falling three-fold dilutions of the various drugs were used and 0.2 c.c. was placed in each small test-tube. The medium was kept at 50° C. in a water bath and 10 per cent horse blood was added just before distribution, when 1.8 c.c. of the final mixture was added to each test-tube. Care was taken to make a uniform admixture of the substances in the test-tube. The tubes were sloped and left overnight on the bench. They were sown with a loop on the following day with undiluted 24-hour culture and all tests were read after incubation overnight, except when the brucella organism was used.

A strain of *Str. pyogenes* group A was used as a standard in every test against all drugs and very uniform results were obtained. If any drug gave an unsatisfactory end-point in any experiment the result was discarded and a new experiment performed. Most of the strains were tested on several occasions.

Methods employed to record growths in each tube and to calculate an average 50 per cent end-point of inhibition with the respective drug and organism were similar to those adopted by Francis (1952).

In vivo. Chemotherapeutic agents were prepared in the same way as for *in vitro* tests and administered subcutaneously except when otherwise stated. Mice with an average body-weight of 25 g. were selected for experiments. The most virulent strains of *Corynebacterium murium* and *Pasteurella septica* were selected by animal experiments

Mice infected intravenously with 0.2 c.c. of 24-hour culture of *C. murium,* diluted 1 : 4, developed white focal lesions in the kidneys and lungs on the second day and nearly all died on the third day after infection. Treatment was given on the second and fourth days after infection. The days on which mice died were recorded and those surviving on the tenth day were killed and given an arbitrary survival time of 10 days. All mice were subjected to post-mortem and cultural examinations.

A serum broth culture of *P. septica,* isolated from a fowl, killed a proportion of mice when 2×10^{-7} c.c. of a serum broth culture were given intraperitoneally; 2×10^{-6} c.c. killed all mice. In chemotherapeutic tests mice were infected intraperitoneally with 0.2 c.c. of a 1/50 dilution and treatment was instituted within two hours of the infective dose. The following survival times were given to mice dead on the following days :—

Day		1	2	3
		Survival time, hours		
Dead in:	Morning	14	38	62
	Evening	26	50	74
Survivors - 98 hours				

Results and Discussion

In vitro findings

Streptococci. In five preliminary experiments in which milk agar was compared with Difco meat infusion agar, sulphadimidine gave a 7-fold higher titre against streptococci on milk agar medium than on Difco agar. Similarly, horse blood was compared with sheep blood in four experiments, and a 9-fold higher titre was obtained with horse blood. The effect of the different bloods was similar in milk agar and in infusion agar media. By using both milk agar and horse blood, *Str. pyogenes* was inhibited by sulphadimidine at an average concentration of 9.3 μg./ml., which is similar to the effective *in vivo* concentration.

Twenty strains of streptococci isolated from clinical cases of strangles and rhinitis in horses, some *Str. equi* and some *Str. zooepidemicus,* were examined. Sixteen were inhibited by 0.02 to 0.08 μg./ml. and the remaining four strains by 0.006 μg./ml. of penicillin. All strains were moderately resistant to chlorotetracycline and oxytetracycline, but their responses to both of these antibiotics were similar except for individual strain variations (Love, *et al.,* 1954, and Ransmeier, *et al.,* 1951). Seven strains were quite resistant to sulphadimidine, but the remainder were inhibited, on an average, by 94.83 μg./ml. of sulphadimidine. There was no regular correlation between bacterial type and sensitivity. It will be noted that the strains isolated from the clinical case of horses were, on average, 10 times more resistant to penicillin and sulphadimidine sodium, and four times more resistant to other antibiotics tested, than the group A streptococcus. This higher resistance may explain the rather poor results obtained in the treatment of strangles in the horse (Bryan, 1946; Minett and Edwards, 1945; Niemand, 1943; Roberts 1945; and Sheetz, 1951).

Corynebacterium pyogenes. Seven strains of *C. pyogenes,* isolated from clinical cases, were highly sensitive to penicillin, being inhibited by an average of .002 μg./ml. They were sensitive to the other antibiotics and to sulphadimidine (Coles, 1948; Edwards, 1952; Morse and Spencer, 1950) except for one strain which was quite resistant to sulphadimidine. In the chick-embryo Francis (1947) found that one strain of *C. pyogenes* did not respond at all to treatment with sulphadimidine, but it was sensitive to penicillin *in vitro,* in the chick-embryo and in mice. *C. pyogenes* was more susceptible to penicillin than the other Corynebacteria.

Corynebacterium equi. Twenty-three strains of *C. equi,* some isolated from clinical cases and some from lesions of pseudotuberculosis in pigs (Tammermagi, 1953), were uniformly resistant to penicillin. So far as it is known, this has not been recorded previously. It is evident also from the table that *C. equi* was more resistant to all drugs except streptomycin and chloromycetin than were the other corynebacteria (Bond, *et al.,* 1952; Brennan and Lasky, 1954).

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TABLE 1

INHIBITORY ACTION OF DRUGS IN MILK MEDIUM CONTAINING HORSE BLOOD

Concentrations (μg/ml.) causing 50% inhibition after incubation over-night at 37°C.

ORGANISMS	NO. OF STRAINS	NO. OF TESTS	PENICILLIN		CHLOROTETRA-CYCLINE		OXYTETRA-CYCLINE		CHLORAM-PHENICOL		STREPTOMYCIN		SULPHA-DIMIDINE	
			Range	Aver-age	Range	Aver-age	Range	Aver-age	Range	Aver-age	Range	Aver-age	Range	Aver-age
Str. pyogenes group A	1	20	-	.002	-	2.2	-	2.0	-	1.5	-	8.7	-	9.3
Str. equi & Str. zoosepi-demicus	30	28	.002-.08	.03	4.1-40.1	19.2	4.2-40.6	18.3	-	-	-	-	8.0-160.0[E]	94.8[E]
C. pyogenes	7	10	.006-.01	.002	1.4- 8.9	4.4	1.5- 4.4	2.7	1.2- 6.6	1.3	.74- 2.2	1.6	5.9-17.8[F]	10.5[F]
C. ovis	4	9	.02 -.08	.06	1.4- 8.9	3.5	1.4- 3.2	2.6	.54- 1.6	1.5	.74- 4.8	2.5	5.8-17.8	7.8
C. murium	7	10	.08-.74	.27	4.1-12.3	8.5	1.3- 9.6	3.4	1.2- 2.2	1.6	1.6- 4.8	2.3	>1000	>1000
C. renale	1	4	-	.14	-	8.9	-	8.6	-	2.4	-	2.2	>1000	>1000
C. equi	23	25	>9	>9	26.7-80.2	36.3	40.0-86.7	58.9	4.9-10.6	7.7	1.6- 4.8	2.7	17.8-53.4	30.1
Species of Corynebact-erium														
C. koala[A1]	4	4	>9	>9	37.0	37.0	13.3-40.0	24.0	1.6- 4.9	3.7	1.6- 4.8	2.5	17.8-74.0	51.9
C. koala[A2]	3	3	>9	>9	37.0	37.0	13.3-40.0	25.9	8.5-10.6	8.7	60.0	60.0	53.4-222	165
Ery. rhusio-pathiae	4	6	.02-.24	.07	4.1-12.3	9.3	4.4-12.4	7.1	4.9-14.7	10.3	2.2-60.0	38.7	>1000	>1000
Br. abortus[B]	1	3	-	.07	-	1.3	-	.62	-	1.6	-	3.3	-	148
P. septica	9	13	.03-.24	.17	4.1-37.0	7.7	3.0-12.5	5.4	1.6-4.9	2.8	4.8-20.0	13.4	24.5-160	47.8
Salmonella spp.[C]	4	6	>9	>9	12.3-80.2	40.9	4.2-37.5	20.1	4.8-14.4	8.0	6.7-42.2	37.8	17.2-160	44.7
S. pullorum	1[D]	3	>9	>9		10.6		7.0		1.4		17.1		21.2
	1	3				58.6		18.5		2.1		40.2		8.2
S. choleraesuis	1[D]	3	>9	>9		12.3		8.4		1.6		17.4		24.7
	1	3	>9	>9		61.7		23.7		3.0		41.7		9.6
S. dublin	1[D]	3	>9	>9		24.7		21.1		3.4		31.7		197
	1	3	>9	>9		160		160		71.1		160		63.7

A Strains isolated from koala bears, A1 before treatment, and A2 after treatment with streptomycin.

B Incubated for 72 hours in 10% carbon dioxide.

C S. amstum, S. potsdam, S. eastborne, S. bonareinsis

D Difco-meat extract agar used as basis instead of milk agar.

E Excluding 7 strains resistant to 1000 μg. per ml.

F " 1 strain " " " " " "

Corynebacterium "koala." The main properties of four strains of corynebacteria isolated from an outbreak of rhinitis and pneumonia in koala bears are shown in Table 2. In addition, all strains failed to ferment salicin, starch, inulin, arabinose, trehalose or glycogen. They produced pinhead colonies on

TABLE 2

Biochemical Reactions of Two Strains of Corynebacteria

Species	Motility	Production of Acid from:					Indole	Urea	Liquefaction of Gelatine	Production of Haemolysin	Oxygen Requirements	Pathogenicity	Toxin Production	Liquefaction of Dorset's egg	Litmus Milk
		Dextrose	Maltose	Saccharose	Lactose	Mannitol									
C. murium	-	+	+	+	-	-	-	+	-	-?	A & F AN.	+	+?	-	-
C. "koala"	-	-	-	-	-	-	+	+	-	-?	A & F AN.	-	-	-	ALK.

infusion or blood agar after 24 hours, which became larger and mucoid after further incubation. The growth was, therefore, very similar to that of *C. equi* except that it did not become pigmented, even after a month on Dorset's egg medium. The organism grew vigorously in infusion broth, producing a diffuse turbidity and strings of mucoid growth. Organisms were poorly Gram-positive, but showed diphtheroid grouping; no acid-fast forms were observed. It will be seen (Table I) that the response of the strain isolated from the koala bear, to different therapeutic drugs, was almost the same as that of *C. equi.*

The outbreak in the koala bears was discovered on April 1, 1954; the animals showed thick muco-purulent discharge and failed to respond to aureomycin treatment. When the causative organism was known to be a corynebacterium, and was found to be highly sensitive to streptomycin *in vitro,* the affected bears were treated on April 22 with 200 units of streptomycin given intramuscularly for five days. Saline (containing 200 units of streptomycin per c.c.) was sprayed into each nostril twice daily for five days. All responded well to treatment and recovered, but koala No. 2 showed further nasal discharge on May 6 and was sprayed with 500 units of streptomycin daily for a week. It appeared to be normal after treatment but on June 7 it suddenly became ill again with a cough and a copious, thin nasal discharge, and died. Post-mortem examination showed intense pulmonary œdema and some emphysema; numerous corynebacteria were isolated from the lungs. It will be seen that three strains isolated after treatment were much more resistant to streptomycin than before treatment, and somewhat more resistant to sulphadimidine, although their responses to chlorotetracycline and oxytetracycline remained the same (Kanegis, *et. al.,* 1950; Nichols and Nidham, 1949; Paine, *et al.,* 1948, 1949).

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Corynebacterium murium (*C. kutscheri*). *C. murium* was isolated from an outbreak of disease in a laboratory stock of mice. This organism had been responsible for intermittent deaths for at least six months before it decided to kill the remaining 135 mice in the colony. When this was done, white focal lesions were found in 13 mice, and they were distributed as shown below:—

Liver, 9; lung, 1; kidney, 1; spleen, 1; lumbar lymph-nodes, 3; submaxillary lymph-node, 1.

The main properties of eight strains of *C. murium* are shown in Table 2. They produced a diffuse turbidity in broth and growth on agar resembled that of *C. ovis*. The organisms were Gram-positive and had a diphtheroid appearance. The seven strains tested were, on the average, inhibited by 0.27 μg. of penicillin per ml.; their responses to other antibiotics were similar to those of other corynebacteria, but they were resistant to sulphadimidine (Table I).

Corynebacterium renale. Infections with *C. renale* are said to respond well to penicillin treatment (Arthur, 1949; Beck, *et al.*, 1945; Fox, 1947; Kiesel, *et al.*, 1947; Morse and Spencer, 1948), but Lovell and Cotchin (1947) recommended early treatment for complete recovery, and found considerable variation in the sensitivity of strains. The strain included in these experiments was inhibited by 0.136 μg./ml. of penicillin. This strain, like *C. murium*, was resistant to sulphadimidine, but it was very sensitive to chloromycetin and streptomycin.

Corynebacterium ovis. Four strains of *C. ovis*, isolated from clinical cases, were very sensitive to penicillin and other antibiotics, and were inhibited by sulphadimidine at a concentration of 7.79 μg./ml., as was found also by Maclay and Slavin (1947). Fontanelli and Casteluchi (1951) found that penicillin reduced the mortality in experimental guinea-pigs from 100 per cent to 40 per cent.

Salmonella. Out of eight strains of salmonella organisms tested, *S. choleræ-suis* and *S. pullorum* were the most sensitive to sulphadimidine, being inhibited by 9.6 μg./ml. and 8.2 μg./ml. respectively. Both the strains were more resistant to streptomycin than to sulphadimidine on the milk agar, but when tested on infusion agar they were much more sensitive, as found by Coles in tryptose blood agar medium. However, it is evident from Table 1 that all strains of salmonella were more susceptible to chloromycetin than to the other drugs. *S. dublin* was uniformly resistant to all antibiotics in milk medium, but moderately sensitive on infusion medium. With sulphadimidine the results were reversed. All the strains of salmonella were resistant to penicillin (McLean, *et al.*, 1949; Schneierson, 1952).

Although streptomycin has some effect *in vitro* against salmonella species, it proves unsatisfactory in experimental trial, and in clinical cases. Schaaf (1953) treated 25 cows suffering from severe gastro-enteritis due to *S. dublin*, with chloramphenicol intravenously: 20 cows recovered immediately after treatment, three recovered after repeated treatment and two died. All the cows which recovered were not bacteriologically sterile. Ben David (1953), using chlorotetracycline orally, and Brennan and Lasky (1954) oxychlocycline intravenously, in

calves infected with *S. dublin* obtained very satisfactory results (Pulaski and Armpacher, 1947).

Sulphonamide therapy of bacillary white diarrhœa of chicks (*S. pullorum* infection) only reduces the mortality (Bottorff and Kiser, 1947; MacNamee, 1947; and Pomeroy, *et al.*, 1948), but Smith (1954) found that furazolidone at a concentration of .04 per cent in the mash was superior to chloramphenicol and sulphamethylpyramidine in experimental *S. pullorum* infection in chicks, and recovered chicks were bacteriologically sterile.

Brucella abortus. One strain of brucella-like organism (brepp-25), isolated by Simmons and Hall (1953) from the epididymis of a merino ram suffering from orchitis, was very sensitive to chlorotetracycline, oxytetracycline, chloramphenicol and streptomycin (Yow and Spink, 1949). Many strains of *Brucella abortus* are resistant to penicillin (Hodgson, 1945), but the brucella-like organism was inhibited by 0.07 μg./ml. of penicillin, as found by Simmons and Hall. It was inhibited by 148.16 μg./ml. of sulphadimidine.

Therapeutic trial with sulphonamides and streptomycin against experimental brucellosis in animals has not been successful. Treatment with chlorotetracycline in pigs naturally or artificially infected with *Brucella suis* yielded some evidence of effect (Burnell, *et al.*, 1953), but Larson and Gilman (1950) could not bring about a complete cure by treating cows suffering from brucellosis. Carle and Larson (1949), however, found that the addition of streptomycin to chlorotetracyline increased its effect against experimental brucella infection in guinea-pigs.

Erysipelothrix rhusiopathiæ. Four strains of *Erysipelothrix rhusiopathiæ* were very sensitive to penicillin, being inhibited by an average concentration of 0.074 μg./ml. (Lawrence and Francis, 1953; Prier and Alberts, 1950; Ullberg, 1953; Verge, *et al.*, 1950); moderately sensitive to aureomycin, 9.27 μg./ml.; terramycin, 7.27 μg./ml.; chloromycetin, 10.33 μg./ml.; but relatively resistant to streptomycin (Katic, 1951; Verge, *et al.*, 1950). All the strains tested in the present experiments were resistant to sulphadimidine, a result similar to that obtained by other workers.

Penicillin has proved most successful in treating experimental infection in mice (Heilman and Herrel, 1944; Woodbine, 1947, 1948, 1950) and natural infection in pigs and man (Aitkin, 1949, Brown, 1949; MacDougall, 1951). A dosage rate of 30 mg. of chlorotetracycline per kg. protected 100 per cent of experimentally infected mice (Little, 1949), but there is no information on its value in the field. Streptomycin is less effective, but proved synergistic with penicillin in experimental infection in mice. It has been shown (Francis, 1947; Slavin and Maclay, 1947; Woodbine, 1946) that sulphonamides have no action against *E. rhusiopathiæ* infection in mice.

Pasteurella septica. A strain of *P. septica* isolated from fowls was considerably more resistant to sulphadimidine than *Str. pyogenes,* as were eight other strains isolated from clinical cases in a variety of animals in Queensland. They

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were moderately sensitive to penicillin and other antibiotics. Morse found that there was great variation in susceptibility depending on the medium used, thus five strains of *P. septica,* which were inhibited in blood agar by 1.1 μg./ml. of terramycin, required for their inhibition in skim milk more than 100 μg./ml. Francis found a strain of *P. septica* isolated from a calf in England more sensitive to sulphadimidine than streptococci. These large variations in the susceptibility of strains of *P. septica* to various drugs have been reported by a number of workers (Neter, 1950, 1951).

In Vivo Findings

Corynebacterium murium.

TABLE 3

Effect of Treating Mice Infected Intravenously with Corynebacterium murium

Drug	Dose, mg., s/c	No. of Mice	Av. Survival Time in Days (max. - 10 days)	Percent. of Survivors	Percent. of Mice Free from Lesions	Percent. of Mice Free from Bacteria on Culture
Procaine Penicillin G.	20	18	4.0	0	0	0
Chloramphenicol	4	18	5.0	23.5	17.7	23.5
Chlorotetracycline	1	18	8.0	61.9	23.8	47.6
Oxytetracycline	8	18	8.5	77.8	38.9	66.6
Streptomycin	8	18	9.5	94.1	91.1	100.0
Control	Nil	18	3.0	0	0	0

It will be seen from Table 3 that untreated mice infected with *C. murium* died after an average period of three days. All mice that died, either in the control group or in the treated groups, had white foci in the lungs and kidneys, and a few of them had some of these lesions in the mycocardium. It is evident from Table 3 that 20 mg. of procaine penicillin had only a slight beneficial effect on the course of the disease. Chloramphenicol at a dose of 6 mg. per mouse protected only 17 per cent of the mice. Chlorotetracycline and oxytetracycline had a better effect and the latter protected more than 77 per cent of the infected mice. Streptomycin protected 94 per cent and though a few of them when killed had some lesions in their internal organs, all proved sterile on culture. In two subsequent experiments, increasing the dose of chlorotetracycline to 8 mg. only slightly increased its effect. The effects of different antibiotics against experimental *C. murium* infection in mice are similar to those with *C. diphtheriæ* infection in guinea-pigs (Hewitt, 1948, 1950, 1952).

Pasteurella septica. All mice infected with *P. septica* which did not receive treatment, died the following morning. The number of mice given the various doses and the mean survival times are shown in Table 4. Statistical analysis showed that the regressions of mean survival times on log (dose) showed no

significant departures from linearity and the linear regression coefficients were homogeneous. The average value of the linear regression coefficient was 43.08 hours/unit increase in log (dose) or 12.97 hours between successive doses. The observed means are summarised in Table 4.

TABLE 4

Effect of Treating Mice Infected Intraperitoneally with Pasteurella septica

Drugs	No. of Mice	Dose (mg.)	Mean Survival Time (hrs.)
Sulphadimidine	13	4	25.1.
	13	8	35.2
	13	16	38.6
Penicillin	13	4	17.3
	13	8	35.9
	13	16	55.1
Chloramphenicol	13	2	18.5
	13	4	34.8
	13	6	42.1
Streptomycin	13	2	38.0
	13	4	43.5
	13	8	67.5
Oxytetracycline	13	1	38.0
	13	2	42.2
	13	4	67.1
Chlorotetracycline	13	.25	48.2
	13	.5	57.4
	13	1	70.3

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Using sulphadimidine as a base, the relative potencies and the corresponding 95 per cent fiducial limits were as follows: Sulphadimidine, 1.0; penicillin, 1.2, 0.5-2.9; chloromycetin, 1.9, 0.8-4.6; streptomycin, 4.9, 1.9-12.4; terramycin, 9.5, 3.8-23.8; chlorotetracycline, 63.0, 24.0-165.2. It will be seen from Table 4 that approximately equal survival times were produced by all drugs except sulphadimidine and chloramphenicol. Procaine penicillin, however, had to be given in very large doses, and chlorotetracycline produced the best result with much the smallest dose, a result similar to that obtained by other workers (Prier, 1950; Soltys, 1951). The poor results with chloramphenicol were surprising, but were not improved when suspensions were made in propylene glycol, or when the drug was given orally.

We gave only treatment, but it has been found that treatment with chlorotetracycline over a period of six days did not substantially increase the survival rate as compared to a single dose on the day of infection. Again, high initial blood levels obtained by Klein, *et al.* (1950) after intravenous administration of 2 mg. of chlorotetracycline did not increase the rate of destruction of Type I pneumococci in the blood stream over that of lower blood levels obtained by giving 0.5 mg. by the intramuscular route. Smith, *et al.* (1948) observed that chloramphenicol is rapidly excreted by mice. Gruhzit, *et al.* (1949) found that by giving 100 mg./kg. of chloramphenicol to mice twice daily a blood concentration of only 11 μg./ml. was obtained, whereas Burnell, *et al.* (1951) concluded, from their experimental findings in man, that 10 to 20 μg./ml. would be an effective concentration in severe infections. The high percentage of survival in the chloramphenicol treated group of mice obtained by Soltys may be due to the use of minimal infective doses, for the control group died only after five days. Similarly, Coles found that streptomycin given to mice one hour after infection with a particular strain of *P. septica,* protected 100 per cent of the infected mice, but with another more virulent strain of *P. septica* even 4 mg. of streptomycin did not give any protection.

This emphasises the importance of strain specificity within the *P. septica* group, which may be one of the factors responsible for the variable clinical results obtained by various workers while treating pasteurellosis in different species of animals with different drugs, particularly with sulphonamides (Delaplane and Higgins, 1948; Hilbert and Kiser, 1948; Kiser, *et al.,* 1947; McAuliff, 1947; Peterson, 1948, Shanks, 1941; 61st Report, 1948; Volt, 1948).

The series of experiments carried out on *P. septica* show the remarkable differences that may be obtained between relative potencies *in vivo* and *in vitro*. Thus, if the dose of penicillin required to produce a given effect is allotted a value of "1," the following relative amounts of other drugs are required *in vivo* and *in vitro*:—

	Penicillin	Chlorotetra-cycline	Oxytetra-cycline	Chloramphenicol	Streptomycin	Sulpha-dimidine
In vitro	1	45.3	31.7	16.5	78.8	281.2
In vivo	1	0.058	0.25	-	0.47	1.6

Similar big differences between the relative *in vivo* and *in vitro* potencies have been recorded with the sulphonamides and are doubtless partly due to the rate of absorption and excretion. On the other hand, types and *pH* of media (Table I) number and growth phase of test organism, all modify the action of drugs, especially chlorotetracycline *in vitro*. Klein (1950) observed that the concentration of chlorotetracycline required to inhibit the growth, *in vitro,* of a Type I pneumococcus after 18 hours of incubation at 37° C. is 5 to 10 times greater than the concentration required to inhibit the growth *in vivo*. Considering the relative potency of chlorotetracycline against Type I pneumoccoccus *in vitro* and *in vivo,* Klein, *et al.,* concluded that only one-fifth to one-tenth of the inhibiting *in vitro* concentration is effective *in vivo*. Edwards found that penicillin was ten times more potent in broth than chlorotetracycline against streptococci, but 10 times less effective in mice. In milk he found that penicillin was 1,000 times more potent than streptomycin against staphylococci, but they had about the same potency in the mouse. *Bact. coli* was slightly susceptible to chlorotetracycline or streptomycin *in vitro,* but responded well to both of these drugs in the mouse.

Unfortunately, our knowledge of the blood concentrations produced by different antibiotics in various species of animals is still limited, whereas these are well known with sulphonamides, particularly in mice. It is therefore easy to calculate relative antibacterial potencies *in vivo* for sulphonamides, but not for the antibiotics. For instance, the average sulphonamide coefficient of sulphathiazole against *Bact. coli in vitro* is 50, whereas the highest *in vivo* coefficient, after corrections have been made for differences in blood concentration, is only 13.

Summary and Conclusions

1. Eighty-eight strains of organism isolated from different clinical conditions in animals and birds and comprising some 15 species, were tested for their sensitivity to different drugs in milk agar medium containing horse blood.

2. The natural diseases produced by *C. murium* in mice and *C. "koala"* in koala bears are described briefly. All bacteria, except *C. equi, C. "koala"* and Salmonella spp., were sensitive to penicillin and they are arranged, according to their sensitivity to penicillin, in the following descending order:—

Str. pyogenes, C. pyogenes, Str. equi and *Str. zooepidemicus, C. ovis, Ery. rhusiopathiæ,* brucella-like organism, *P. septica, C. renale* and *C. murium.*

Equine strains of streptococci were, on the average, 10 times less sensitive to sulphadimidine, and all the antibiotics tested, than was the group A strain. Seven of the 20 strains were resistant to sulphadimidine.

3. The antibacterial spectra of chlorotetracycline and oxytetracycline *in vitro* were very similar, but oxytetracycline was slightly more active than chlorotetracycline against all organisms *in vitro* except *C. equi.* The organisms had the following descending order of sensitivity:—

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Brucella-like organism, *Str. pyogenes, C. ovis, C. pyogenes, Ery. rhusiopathiæ*, salmonella species.

4. All the antibiotics were much more active against salmonella organisms in infusion medium than in the milk agar medium, whereas the reverse was true with the sulphonamides.

5. Chloramphenicol gave the most uniform results against all organisms tested and none were resistant. It would appear to be the best drug for salmonella infections.

6. Different species of corynebacteria were uniformly sensitive to streptomycin and chloramphenicol, but not to other drugs. Responses of strains isolated from the clinical cases of pneumonia in koala bears, before and after treatment with streptomycin, showed the development of a high resistance to streptomycin.

7. *C. murium* infection in experimental mice, as well as the *C. "koala"* infection of koala bears, responded best to streptomycin therapy, but the emergence of the resistant type during treatment would appear to be the greatest barrier against its clinical use. Penicillin had very poor activity, but it will be noted that *C. murium* is much more resistant to penicillin than *C. pyogenes*.

8. In experimental infection with a virulent strain of *P. septica* in mice, chlorotetracycline proved, on the basis of a single dose therapy, to be much the most potent chemotherapeutic agent against this infection. The results with chloramphenicol were very disappointing and did not improve when the drug was given orally, or subcutaneously in propylene glycol. It appears that chlorotetracycline might well be the most active drug against *P. septica* infections.

9. It is evident that, as with the sulphonamides *in vitro*, activity does not necessarily give a good guide to *in vivo* activity. This is doubtless partly due to varying distribution of drugs, but it has been shown that both absolute and relative activities vary considerably in different media.

Acknowledgments

I am indebted to Mr. J. Saal and Miss J. Gray for technical assistance; to Dr. E. W. Moodie and Mr. P. B. McGovern for the statistical analysis. I am also deeply indebted to Professor J. Francis for the help and encouragement received during the experimental work, and in the preparation of the manuscript and for innumerable valuable suggestions.

REFERENCES

Aitken, W. A. (1949) : *N. Amer. Vet., 30,* 25.
Arthur, G. H. (1949) : *Vet. Rec., 61,* 257.
Beck, J. D., Mott, De T., and Boucher, W. B. (1945) : *Univ. Penn. vet. ext. Qt., 100,* 10.
Ben David, B. (1953) : *Refuah. Vet., 10,* 62.
Bond, E., Lebeau, L. J., and Novak, M. (1952) : *Cornell Vet., 42,* 207.
Bottorff, C. A., and Kiser, J. S. (1947) : *Poult. Sci., 26,* 335.
Brennan, F. M., and Lasky, H. H. (1954) : *J. Amer. vet. med. Ass., 124,* 384.
Brown, K. E. (1949) : *Vet. Rec., 61,* 874.
Brownlee, G. (1945) : *Ibid., 57, 229.*
Bryan, A. H. (1946) : *J. Amer. vet. med. Ass., 109,* 57.
Bunnell, D. E., Bay, W. W., and Hutchings, L. M. (1953) : *Amer. J. vet. Res., 14,* 160.
Burnell, J. M., and Kirby, W. M. M. (1951) : *J. Lab. Clin., 38,* 234.

XUM

Carle, B. N., and Larson, C. L. (1949): In "Brucellosis," p. 155. Americal Association for the Advancement of Science, Washington.
Coles, E. H., Jr. (1948): *Amer. J. vet. Res., 9,* 152.
Delaplane, J. F., and Higgins, T. C. (1948): *Cornell Vet., 38,* 267.
Edwards, S. J. (1949): *J. comp. Path., 59,* 191.
Edwards, S. J. (1952): *Ibid., 62,* 220.
Fontanelli, E. R., and Casteluchi, I. (1951): *Zooprofilassi, 6,* 299.
Fox, F. M. (1947): *Cornell Vet., 37,* 59.
Francis, J. (1947): *Vet. Rec., 59,* 131.
Francis, J. (1952): *Brit. J. Pharmacol, 7,* 189.
Gruhzit, O. M., Fisken, R. A., Reutner, T. F., and Martino, E. (1949): *J. clin. Invest., 28,* 943.
Heilman, F. R., and Herrel, W. E. (1944): *Proc. Mayo Clin. Staff Mtg., 19,* 340.
Hewitt, L. F. (1948): *Brit. J. exp. Path., 28,* 338.
Hewitt, L. F. (1952): *Ibid., 33,* 217.
Hewitt, L. F. (1950): *J. Path. Bact., 29,* 289.
Hilbert, K. F., and Kiser, J. S. (1948): *Cornell Vet., 38,* 148.
Hodgson, G. A. (1945): *Brit. med. J., 1,* 483.
Jennings, I. W. (1947): *Vet. Rec., 59,* 369.
Kanegis, L. A., Kiser, J. S., Dornbush, A. C., Burkhart, R. L., Gadekar, S. M., de Mello, G. C., and Pelcak E. J. (1950): *Vet. Med. 45,* 313.
Katic, V. R. (1951): *Vet. Glasnik., 5,* 465.
Kiesel, G. K., Morse, E. V., and Evans, W. N. (1947): *Cornell Vet., 37,* 173.
Kiser, J. S., Greene, L. M., Prier, J., and Bottorff, C. A. (1947): *Poult. Sci., 26,* 546.
Klein, M., Schorr, S. E., Tashman, S., and Hunt, Jr., A. D. (1950): *J. Bact., 60,* 159.
Larsen, P. H., and Gilman, H. L. (1950): *Cornell Vet., 40,* 259.
Lawrence, J. S., and Francis, J. (1953): "The Sulphonamides and Antibiotics in Man and Animals," 482. London: H. K. Lewis & Co., Ltd.
Lebeau, L. J., Bond, E., Danicki, J., and Novak, M. (1952): *Cornell Vet., 42,* 200.
Little, P. A. (1949): *Ann. N.Y. Acad. Sci., 51,* 246.
Love, B. O., Jr., Wright, S. S., Purcell, E. M., Moo, T. W., and Finland, M. (1954): *Proc. Soc. exp. Biol. N.Y., 85,* 25.
Lovell, R., and Cotchin, E. (1947): *J. comp. Path., 57,* 20.
Maclay, M. H. L., and Slavin, G. (1947): *Ibid., 57,* 218.
MacNamee, J. K. (1947): *J. Amer. vet. med. Ass., 111,* 295.
Martin, G. (1953): *Wien Tierärztl. Mschr., 40,* 339.
McAuliff, J. L. (1947): *J. Amer. vet. med. Ass., 110,* 314.
MacDougall, J. A. (1951): *Lancet, 1,* 1345.
McLean, I. W., Jr., Schwab, J. L., Hillegas, A. B., and Schlingman, A. S.: (1949): *J. clin. Invest, 28,* 953.
Minett, F. C., and Edwards, W. P. S. (1945): *Indian J. vet. Sci., 15,* 1.
Morse, E. V., and Spencer, G. R. (1950): *Vet. Med., 45,* 405.
Morse, E. V., and Spencer, G. R. (1950): *Ibid., 45,* 221.
Morse, E. V., and Spencer, G. R. (1948): *Cornell Vet., 38,* 135.
Neter, E., Gorzynski, G. A., and Cass, E. A. (1951): *Proc. Soc. exp. Biol. N.Y., 76,* 493.
Neter, E., Gorzynski, G. A., and Cass, E. A. (1950): *Ibid., 74,* 328.
Nichols, D. R., and Nidham, G. M. (1949): *Proc. Mayo Clin. Staff Mtg., 24,* 309.
Niemand, H. G. (1943): *Z. Veterinark, 55,* 29.
Paine, T. F., Jr., Collins, H. S., and Finland, M. (1949): *Ann. N.Y. Acad. Sci., 51,* 228.
Paine, T. F., Jr., Collins, H. S., and Finland, M. (1948): *J. Bact., 56,* 489.
Peterson, E. H. (1948): *J. Amer. vet. med. Ass., 113,* 263.
Pomeroy, B. S., Fenstermacher, R., and Roepke, M. H. (1948): *Ibid., 112,* 296.
Prier, J. E. (1950): *Vet. Med., 45,* 243.
Prier, J. E., and Alberts, J. O. (1950): *J. Bact., 60,* 139.
Pulaski, E. J., and Armpacher, W. A. (1947): *Bull. U.S. Army med. Dept., 7,* 101, 221.
Ransmeier, J. C., Brown, H. E., and Davis, N. (1951): *J. Lab. Clin., 38,* 620.
Roberts, S. J. (1945): *Cornell Vet., 35,* 378.
Soltys, M. A. (1951): *Vet. Rec., 63,* 689.
Scaaf, A. (1953): *Tijdschr. Diergeneesk., 78,* 721.
Schneierson, S. S. (1952): *J. Lab. Clin., 40,* 48.
Shanks, P. L. (1941): *Vet. Rec., 53,* 85.
Sheetz, H. O. (1951): *Vet. Med., 46,* 38.
Simmons, G. C., and Hall, W. T. K. (1953): *Aust. vet. J., 29,* 33.
Sixty-first Annual Report of the South Dakota agricultural experimental station (1948): 48.
Siegal, M., and Julianelle, L. A. (1945): *Ann. intern. Med., 22,* 1.
Slavin, G., and Maclay, M. H. (1947): *J. comp. Path., 57,* 209.
Smith, H. W. (1954): *Vet. Rec., 66,* 493.

XUM

Smith, R. M., Joslyn, D. A., Gruhzit, O. M., McLean, I. W., Jr., Penner, M. A., and Ehrlich, J. (1948) : *J Bact., 55,* 425.
Tammemagi, L. (1953) : *Qld. J. agric. Sci., 10,* 81.
Ullberg, S. (1953) : *Nord. vet. Med., 5,* 677.
Verge, J., Goret, P., Jobert, L., and Cauchy, L. (1950) : *Ann. Inst. Pasteur, 79,* 145.
Volt, H. M. de (1948) : *Amer. J. vet. Res., 9,* 215.
Watts, P. S., and McLeod, D. H. (1946) : *J. comp. Path., 56,* 170.
Woodbine, M. (1946) : *Vet. J., 102,* 88.
Woodbine, M. (1947) : *Ibid., 103,* 149.
Woodbine, M. (1948) : *Brit. med J., 1,* 404.
Woodbine, M. (1950) : *Bact. Rev., 14,* 161.
Yow, E. M., and Spink, W. W. (1949) : *J. clin. Invest., 28,* 871.

NEWS

Dr. R. F. Gordon Honoured

A DISTINGUISHED audience which included representatives from the poultry industry, veterinary profession and Ministry of Agriculture, gathered at Grosvenor House, London, on Thursday, March 7, to witness the presentation of the 1956 B.O.C.M. Poultry Award "Noble Prize" to Dr. R. F. Gordon.

Dr. Gordon is Director of the Houghton Poultry Research Station, Hunts (formerly under the Animal Health Trust) and he has done a great deal of fundamental research into poultry diseases and associated problems.

During the presentation ceremony, Mr. Guy Chipperfield, C.B.E. (Chairman, B.O.C.M.) remarked that this was the fourth occasion on which he had presented this annual award to the representative of the poultry industry who, in the opinion of an independent selection committee, had made the greatest contribution to the welfare of the industry.

Mr. Chipperfield said: "I always feel very much at home with people connected with the poultry industry. They always show a spirit of enterprise and progress, and there exists amongst them a very real appreciation of how science and practice can be combined to the benefit of all."

There was still room for progress within the industry. A comparison of the amount of poultry meat consumed in the U.S.A. and this country offers a challenge to our home producers. He considered that a saving in imports of meat, while at the same time bringing poultry products within the reach of a much greater proportion of the population, was a worthwhile proposition. Great progress had been made in refrigeration equipment which facilitated processing, and organisations were in being for marketing poultry meat in forms which were both appetising and reasonably cheap in price.

In paying tribute to Dr. Gordon, Mr. Chipperfield stressed the great benefit to the poultry industry which resulted from a close liaison between official research workers and the primary producer. Dr. Gordon personifies this excellent spirit of co-operation. Since he graduated as a member of the Royal College of Veterinary Surgeons in July, 1933, he has shouldered increasing burdens of responsibility in an ever-widening field of endeavour. The thanks of the whole poultry world are due to Dr. Gordon for his outstanding work and contribution to its prosperity.

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